Infection and Cardiovascular Disease:
The Atherosclerosis Risk in Communities Study

A DISSERTATION
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Logan Thomas Cowan

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Kamakshi Lakshminarayan MD, PhD, Advisor

July 2017

Acknowledgements

I am grateful to have associated with such incredible students and faculty at the University of Minnesota. I would like to first thank my advisor, Dr. Kamakshi Lakshminarayan for being approachable and encouraging me to become an independent researcher. I would also like to thank my dissertation committee, Dr. James Pankow, Dr. Pamela Lutsey, and Dr. David Vock for their input and feedback on my work and their support of my ambitious timeline. I have benefited greatly from my association with Dr. Aaron Folsom. His support of student projects and his responsiveness to students are unparalleled. I am also grateful for the support that I have received under his T32 training grant (T32HL007779). I would also like to thank my MPH advisor at Brigham Young University, Dr. Brianna Magnusson for teaching me the foundations of epidemiology and encouraging me to consider doctoral training. I would also like to thank the staff and participants of the ARIC study for making this work possible.

I am grateful for the CVD fellows and my PhD cohort for their friendship. I would like to specifically thank Rachel Ogilvie for her constant encouragement and advice throughout my PhD experience. I would also like to thank Faye Norby for sharing her profound knowledge of ARIC data and SAS.

I am forever grateful for the love and support of my parents, Tom and Lori Cowan. They taught me the value of education and make tremendous sacrifices to make education possible for our family. My siblings have been a perpetual source of support. My brother Ben showed me that it is possible to simultaneously be an excellent student, scholar, husband, and father. His passion for education is contagious, returning to

graduate school did not seem so daunting because of his confidence in me. I have always tried to be like him and am a better man as a result.

Finally, and most importantly, I would like to thank my beautiful wife Kate, she has been a constant source of support and encouragement. Her unselfishness towards me and my academic pursuits defy description. She has also blessed us with four wonderful children, Carli, Janie, Milo, and Tad. Their companionship and love have made this journey much sweeter.

Dedication

This dissertation is lovingly dedicated to my wife Kate, the greatest champion of my academic dreams.

Abstract

Infection has been identified as both a chronic and acute risk factor of cardiovascular disease (CVD). Despite the growing body of evidence, additional research elucidating the relationship between infection and CVD is needed. This dissertation employs longitudinal data from the Atherosclerosis Risk in Communities (ARIC) study, the Longitudinal Investigation of Thromboembolism Etiology (LITE) ancillary study, the Dental-ARIC (D-ARIC) ancillary study, and the corresponding ARIC study participant Centers for Medicare and Medicaid Services (CMS) data to examine the relationship between infection and CVD.

In the first manuscript we assessed the longitudinal relationship between self-reported periodontal disease and clinical periodontal disease and incident venous thromboembolism (VTE). Self-reported periodontal disease was associated with 30% higher VTE risk that remained significant or borderline significant after adjustment. Crude associations between clinical periodontal disease classifications were attenuated with adjustment and were no longer significant.

In the second manuscript we assessed the longitudinal relationship between history of endodontic therapy (ET) and incident coronary heart disease (CHD), ischemic stroke, heart failure, and VTE. We found no significant associations between self-reported history of ET and any of our outcomes of interest that remained after adjustment.

In the final manuscript we used a case-crossover study design to evaluate infection as a potential trigger of CHD, ischemic stroke, and VTE. Infection was associated with

higher odds of CHD, stroke, and VTE up to 90 days following the infection. The association between infection and CVD/VTE was graded such that the infection-CVD/VTE association was highest immediately following the infection and decreased as the time since the infection increased. Generally, outpatient infection was a weaker CVD/VTE trigger compared to all infections.

Further research is needed to pinpoint if periodontal disease is independently associated with VTE risk and if periodontal prevention and treatment could reduce VTE risk. Our results do not support an independent association between endodontic therapy and CVD or VTE. The results of the third manuscript provide evidence in support of our hypothesis that infection is a CVD/VTE trigger. Patients with an infection who are at elevated risk of CVD should be considered potential candidates for CVD prophylaxis during and immediately after infection to reduce the otherwise elevated CVD/VTE risk.

Table of Contents

List of Tables

List of Figures

Chapter 1 – Background

Overview of Cardiovascular Disease

Cardiovascular disease (CVD), including coronary heart disease (CHD) and stroke is the leading cause of death and disease burden worldwide.[1] An estimated 17.5 million people die from CVD annually, representing 31% of all global deaths.[2] In the United States, CVD is the leading cause of death for both men and women accounting for around 610,000 deaths each year.[3] CHD is the most common type of heart disease in the United States and is responsible for killing over 365,000 people annually.[3] CVD is also the largest source of disability and premature death and the leading source of disability adjusted life years (DALYs) for both men and women in the U.S.[4] Nearly 20% of CVD related deaths occur in persons younger than 65 and 35% occur before age 75.[3]

As a result of the health burden associated with CVD, a great deal of epidemiologic research has been devoted to identifying and describing CVD risk factors. Population-based cohort studies have identified many chronic risk factors for cardiovascular disease (CVD) that are both modifiable including high blood pressure, elevated serum cholesterol, and smoking, and non-modifiable including male sex, non-white race, family history, and greater age.[5-7] Further, acute risk factors – or triggers – of CVD events have been identified including physical exertion, stress, sexual activity, alcohol abuse, and drug use among others.[8, 9] Infection has been proposed as both a chronic and acute risk factor of CVD.

Infectious Diseases and Cardiovascular Disease

Infection is defined as the entry and development or multiplication of an infectious agent in the body of human or animal.[10] Each year, infections are associated

with an estimated 20.2 million outpatient visits and 3.9 million inpatient visits in the United States.[11] Numerous studies have examined the impact of both chronic and acute infections on CVD outcomes. In their review of infection and stroke evidence, Miller and Elkind conclude that there is evidence that a number of infections can directly cause CVD, including bacterial, fungal, parasitic, and viral.[12] While a comprehensive review of the evidence is beyond the scope of this work, understanding the existing evidence related to infection and CVD is important.

Chronic Infection and CVD

Extensive work has been done to identify associations between chronic infections and CVD including viral infections like cytomegalovirus, herpes, hepatitis C, and HIV, and bacterial infections like chlamydia pneumoniae, osteomyelitis, and helicobacter pylori among others.

The association between cytomegalovirus (CMV) and CHD has also been meta-analyzed. Ji et al found that people exposed to CMV infection had 1.67 times the odds of CHD compared to unexposed persons.[13] Herpes simplex virus (HSV) infection has also been studied as a CVD risk factor in a meta-analysis. Wu et al found that participants exposed to HSV-1 infection exhibited an increased risk of atherosclerosis (OR = 1.77) while HSV-2 positive participants also demonstrated significantly increased athersclerosis risk (OR = 1.37).[14] The impact of hepatitis C viral infection (HCV) on CVD was recently assessed in a meta-analysis conducted by Petta et al. They found that HCV-infected patients had increased risk of CVD-related mortality (OR = 1.65) and stroke (OR = 1.30).[15] Another meta-analysis conducted in 2016 by Olubamwo et al found that the risk of developing coronary atherosclerosis among persons with chronic hepatitis

C is about triple compared to uninfected persons (OR = 3.06).[16] Considering HIV, Islam et al conducted a meta-analysis and found that CHD was 61% higher among people infected with HIV compared to uninfected people.[17]

Extensive research and meta-analyses have also been done to evaluate the impact of bacterial infections on CVD. In 2015, Corrales-Medina et al used data from the Atherosclerosis Risk in Communities (ARIC) study to evaluate the impact of hospitalized pneumonia on CVD. They concluded that pneumonia is both an acute and chronic risk factor for CVD.[18] Two prior meta-analyses also found associations between chlamydia pneumoniae and cardiovascular disease. Danesh et al found that the presence of chlamydia pneumoniae IgA titres was associated with a 25% increase in CHD risk.[19] Simlarly, Smieja et al found that the prevalence of circulating chlamydia pneumoniae DNA was associated with a 2-fold increase in CVD.[20] A recent meta-analysis of pneumonia and stroke conducted by Su et al showed that pneumonia infection was significantly associated with a two-fold increase in ischemic stroke risk.[21] Considering osteomyelitis and CHD, Hsiao et al found that the risk of CHD was 1.65 times higher among patients with chronic osteomyelitis compared to control patients.[22] Two recent meta-analyses of studies looking at helicobacter pylori (H. pylori) and CHD have been done. Sun et al found that H. pylori infection increases CHD risk by 11%.[23] Liu et al found that H. pylori is associated with a 2-fold increase risk of myocardial infarction (MI).[24] An earlier meta-analysis conducted by Zhang et al found that H. pylori infection increases CHD risk (OR = 2.11) and ischemic stroke (OR = 2.68).[25] Two more recent meta-analses done byYu et al[26] and Wang et al[27] found a pooled OR=1.97 and OR = 1.60 respectively between H. pylori and stroke.

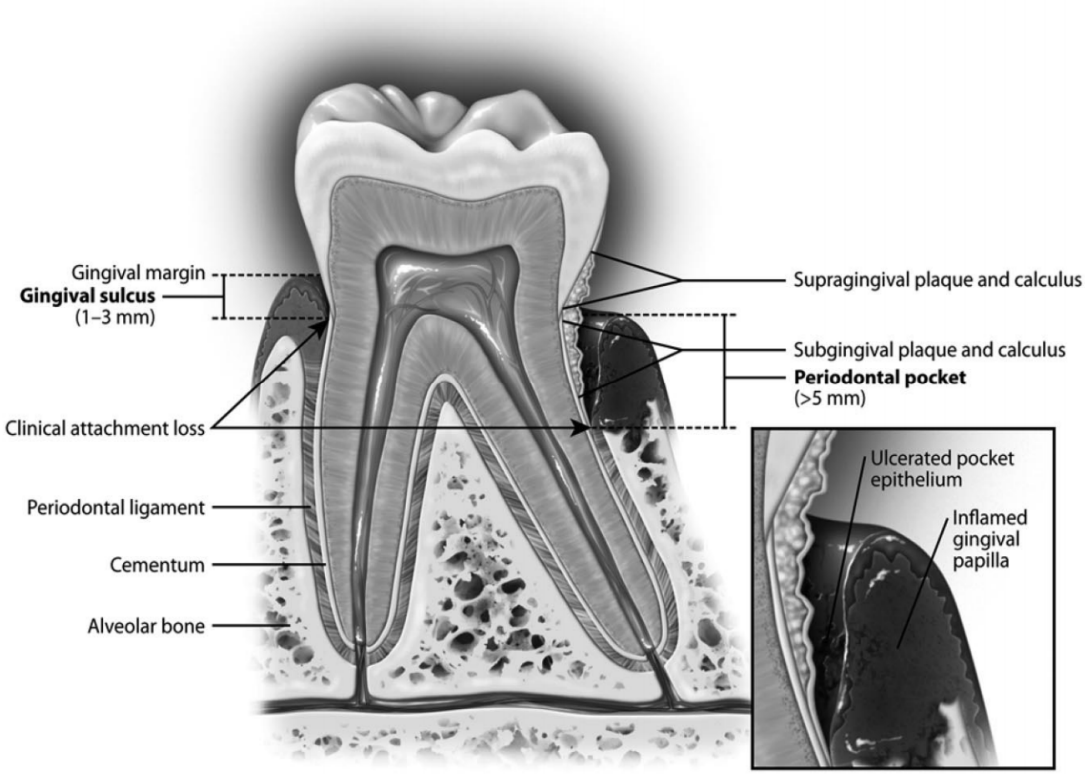
Oral Infections

Among the infections proposed to be associated with CVD are oral infections. Caplan wrote in 2004 that "perhaps the most exciting issue currently facing the dental research community centers around a hypothesized connection between chronic inflammatory oral infections, most notably periodontal disease, and the development of adverse systemic health conditions."[28] The two most prominent dental infectious diseases are periodontal disease (periodontitis) which affects one or more of the periodontal tissues supporting the tooth and endodontic infection in which bacteria infect the tooth's pulp chamber and leads to apical periodontitis or infection of the apex of the tooth root.

Periodontal Disease

Periodontal disease is a chronic inflammatory disease caused by bacterial infection of the supporting tissues around the teeth.[29] Periodontal disease is characterized by inflammatory destruction of the tissues supporting the teeth including gingival tissue, periodontal ligaments, and alveolar bone (see Figure 1 below).[30]

Figure 1. Periodontal disease anatomy and pathology



Symptoms of periodontal disease include bad breath, red or swollen gums, tender or bleeding gums, painful chewing, tooth lose or sensitivity, and receding gums or longer appearing teeth.[31] Treatment options depend on the severity of the disease but all aim to control the infection. Scaling is a procedure that involves removing plaque and tartar from the teeth both above and below the gum line. Root planing gets rid of rough spots on the tooth root where bacteria can gather. Scaling and root planning are used together to prevent and remove the bacteria that contribute to periodontal diease.[31] Antibiotic medications are also used. Surgical procedures are the most comprehensive form of treatment. Flap surgery involves lifting back the gums to remove tartar and then suturing the gums back in place snugly around the teeth. Bone and tissue graft surgery can be performed to help regenerate bone or gum tissue lost as a result of the infection.[31]

Periodontitis is common with around half of all US adults having some periodontitis and nearly 10% having severe periodontitis.[32] Periodontal disease is positively associated with increasing age and is higher among males compared to females.[32] Prevalence is highest in Hispanics (63.5%) and non-Hispanic blacks (59.1%), followed by non-Hispanic Asian Americans (50.0%), and lowest in non-Hispanic whites (40.8%).[32] Prevalence is higher in those of low socioeconomic status (SES) compared to high SES individuals.[32] Other risk factors for periodontal disease include poor oral hygiene, inadequate dental care, cigarette smoking, systemic conditions such as diabetes mellitus, osteoporosis, rheumatoid arthritis, and possibly obesity, stress, and poor coping behaviors.[33] Periodontitis is a significant contributor to tooth loss among adults in the United States.[34]

Periodontal infection has been measured using multiple approaches in past epidemiologic studies. Previous studies of periodontal disease and CVD have used self-reported periodontal disease status ascertained using participant questionnaires.[35] While this is perhaps the easiest and most efficient way to gather exposure data, self-reported disease status can suffer from measurement error since participants may not know their current disease status and past disease information may not be remembered and reported accurately.

Periodontitis has also been assessed using radiography to calculate the bone loss associated with the disease.[36] Another method for measuring periodontal disease is through identifying the presence of systemic antibody titers. Using ARIC data, Beck et al used systemic antibody levels to assess the cross-sectional relationship between

periodontal microbes and coronary heart disease.[37] Similarly, other studies have measured periodontal microbes in periodontal plaque samples.[38]

Clinical examinations have also been frequently used to assess periodontal disease status in the context of studies exploring the association between periodontal disease and CVD. Clinical diagnosis of periodontal disease is based on severity and extent of clinical attachment loss and probing depth. Bleeding upon probing is also frequently used in the clinical assessment of periodontal disease. The most common clinical assessment case definition was created by the Center for Disease Control and Prevention (CDC) Periodontal Disease Surveillance workgroup in collaboration with the American Academy of Periodontology (CDC/AAP).[39]

The CDC/AAP 4-level case definition is as follows:

1. No periodontitis: No evidence of mild, moderate, or severe periodontitis
2. Mild periodontitis: ≥2 interproximal sites (area between teeth) with attachment loss ≥3 mm, and ≥2 interproximal sites with probing depth ≥4 mm (not on same tooth) or one site with probing depth ≥5 mm
3. Moderate periodontitis: ≥2 interproximal sites with attachment loss ≥4 mm (not on same tooth), or ≥2 interproximal sites with probing depth ≥5 mm (not on same tooth)
4. Severe periodontitis: ≥2 interproximal sites with attachment loss ≥6 mm (not on same tooth) and ≥1 interproximal site with probing depth ≥5 mm

Recently, a new clinical periodontal disease classification has been proposed by Morelli et al.[40] It uses 7 tooth-level clinical parameters (interproximal attachment level, probing depth, bleeding on probing, gingival inflammation index, plaque index, the

presence/absence of full prosthetic crowns for each tooth, and tooth status presence) to identify seven distinct periodontal profile classes (PPC) as follows:

1. PPC-A: Health
2. PPC-B: Mild Disease
3. PPC-C: High Gingival Inflammation Index
4. PPC-D: Tooth Loss
5. PPC-E: Posterior Disease
6. PPC-F: Severe Tooth Loss
7. PPC-G: Severe Disease

Periodontal Disease and CVD

Multiple studies have reported an association between periodontal infection and increased risk of coronary heart disease (CHD) and cardiovascular disease (CVD). Previous studies have used a variety of study designs to test whether there is an association between periodontal disease and CVD including cross-sectional,[37, 41-51] case-control,[36, 52-63] and cohort studies.[35, 64-81] Multiple meta-analyses have considered the possible role of periodontal disease in the etiology of CHD.[82, 83] The most recent meta-analysis conducted by Leng et al in 2015 found that among 230,406 participants from 15 studies, periodontal disease was significantly and independently associated with an increased risk of CHD with a pooled RR = 1.19.[83] Earlier meta-analyses also found a significant and independent association between periodontal disease and CHD including Blaizot et al[82] (RR = 1.34), Humphrey et al[84] (RR = 1.24), Mustapha et al[85] (OR = 1.75), Bahekar et al[86] (RR = 1.14), Khader et al[87] (RR = 1.15), and Janket et al[88] (RR = 1.19).

In addition to meta-analyses, recent studies not included in the previous meta-analyses have also identified independent associations between periodontal disease and CVD. In 2016, Hansen et al used data from a Danish nationwide cohort and found that individuals with periodontitis had increased risk of cardiovascular death (IRR = 2.02) and total mortality (IRR = 2.70).[81] Another study published in 2017 by Beukers et al used the electronic health records of 60,174 participants in the Netherlands to study the cross-sectional association between periodontal disease and CVD. They found an independent association between periodontal disease and atherosclerotic cardiovascular diseases (OR = 1.59).[51] The PAROKRANK study recently published results from their case-control study that found an increased risk for a first MI among those with periodontal disease (OR = 1.28) compared to those without periodontal disease.[36]

ARIC data have also been used to study the cross-sectional association between periodontal disease and CHD. Elter et al found that ARIC participants with both high periodontal attachment loss and high tooth loss (OR = 1.5) and edentulous individuals (OR = 1.8) had elevated odds of prevalent CHD compared to individuals with low attachment loss and low tooth loss, while controlling for a number of traditional CHD risk factors.[48] A more recent cross-sectional study using ARIC data performed by Beck et al found that clinical signs of periodontal disease were not associated with prevalent CHD while systemic periodontal antibody response was associated with prevalent CHD.[37]

Previous studies have also explored the potential association between periodontal disease and stroke. In 2012, Sfyroeras et al conducted a meta-analysis and found that the adjusted stroke risk in participants with periodontitis was 1.47 times higher than in

participants without periodontitis.[89]A more recent meta-analysis conducted by Lafon et al found that stroke risk was significantly increased by the presence of periodontitis (RR = 1.63).[90] In 2016, Leira et al published a meta-analysis of 8 studies that found a statistically significant association between periodontal disease and ischemic stroke in both cohort studies (RR = 2.52) and case-control studies (RR = 3.04).[91] Cross-sectional ARIC data have also been used to study the relationship between periodontal disease and stroke. A 2003 paper by Elter et al found that the highest quartile of periodontal attachment loss was associated with prevalent stroke and TIA (OR = 1.3).[92]

Despite the preponderance of existing evidence linking periodontal disease to CHD and stroke, a potential association between periodontal disease and venous thromboembolism (VTE), has received less research attention. Only one previous study considered a possible association between periodontal disease and VTE. Sanchez-Siles et al. used a case-control study design in which 97 patients with VTE and 100 healthy controls were compared for prevalent periodontal disease. They found that periodontitis was more prevalent in VTE patients than controls ($p<.001$).[93] Studies of periodontal disease and CVD published since 2014 are summarized in Table 1 below.

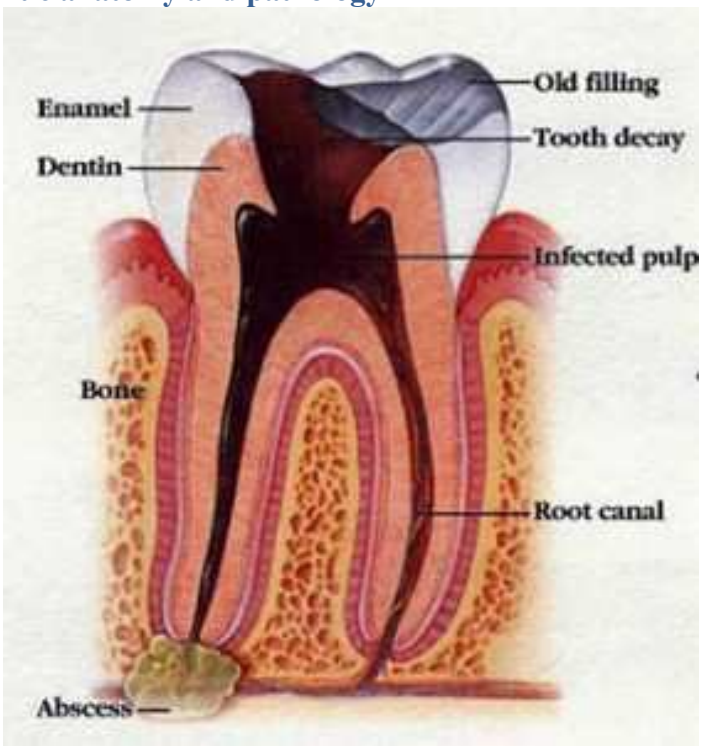
Table 1. Summary of studies on periodontal disease and CVD since 2014

Study	Year	Study Design	Outcome	Association (95% CI)
Hansen et al[81]	2016	Retrospective Cohort	MI	IRR=1.44 (1.30,1.60)
			Ischemic Stroke	IRR=1.89 (1.74,2.05)
			Cardiovascular Death	IRR=2.54 (2.36, 2.72)
			CHD	IRR=2.03 (1.92, 2.14)
			All-Cause Mortality	IRR=3.20 (3.09, 3.32)
Beukers et al[51]	2016	Cross-Sectional	CHD	OR=1.59 (1.39, 1.81)
Rydén et al.[36]	2016	Case-Control	MI	OR=1.29 (1.03, 1.60)
Leira et al [91]	2016	Meta-Analysis	Ischemic Stroke	Cohort RR=2.52 (1.77, 3.58)
				Case-Control RR=3.04 (1.10, 8.43)
Leng et al[83]	2015	Meta-Analysis	CHD	RR = 1.19 (1.13, 1.26)
Lafon et al[90]	2014	Meta-Analysis	All Strokes	RR=1.63 (1.25, 2.00)

Endodontic Infection (EI) and Apical Periodontitis (AP)

Endodontic infection (EI) is caused by bacteria or their byproducts entering a tooth's pulp chamber at the apex of the tooth root.[94] The infected root canal constitutes the main source of persistent microbial irritation to the periradicular tissues which can lead to apical periodontitis (AP), which is the inflammation and destruction of periradicular tissues and infection abscesses surrounding the root end of a tooth (see Figure 2 below).[95, 96]

Figure 2. Endodontic anatomy and pathology



The most common symptoms of EI/AP are pain, tenderness, and inflammation around the infection site but EI/AP can also be asymptomatic.[97] Treatment options for EI/AP include antibiotics which may ease the symptoms but do not typically eradicate the infection.[98] Endodontic therapy (root canal) or tooth extraction is typically required to permanently address EI/AP.[98]

EI/AP is relatively common. Previous studies in western countries have estimated that the prevalence in adults is between 14% and 70%.[28] A recent study conducted in Finland found that 27% of adults had one or more teeth with EI/AP.[99] EI/AP is positively associated with age and is higher among males compared to females.[99] Individual risk factors for EI/AP include poor oral hygiene, inadequate dental care, and cigarette smoking as well as dental risk factors, such as root canal interventions, pulpal posts, inadequate crowns or coronal fillings, primary carious lesions, and reduced marginal bone level.[100]

EI/AP has been measured using multiple approaches in past epidemiologic studies. Clinically, EI/AP is diagnosed through observation of periapical bony lesions on radiographs (indicative of chronic endodontic inflammation) or of radiopaque material in the root canal system (indicative of a history of root canal therapy).[94] Radiographic diagnosis has been used by multiple epidemiologic studies in the past.[101-103] Studies using clinical EI/AP definitions require dental exams and thus often have small sample sizes to accommodate efficient data collection. Researchers often do not have access to participants' radiographs and may be unable to collect radiographs for research purposes due to the cost, required resources, and ethics associated with radiation exposure for research purposes, particularly in large study samples.[28]

In the absence of radiographic information, one way to estimate history of EI/AP is through participants' self-reports of endodontic therapy (ET). Use of self-reported ET as a proxy for EI/AP has limitations.[94, 103] First, ET could be performed for restorative reasons such as prophylaxis in trauma cases and not endodontic reasons. Also, a lack of ET does not necessarily imply the absence of EI/AP since teeth could be extracted or

remain asymptomatic. Further, individuals who have not received or do not have access to dental care may not receive ET when it would be otherwise warranted. Finally, misclassification of exposure could take place since historical ET may be forgotten or mistaken for other procedures.

Despite the limitations, self-reported history of ET has been used as a proxy for EI/AP in the past.[94, 104] Two studies have evaluated the validity of self-reported history of ET compared to radiographic verified ET. In 2002, Pitiphat et al. compared self-reported measures obtained by a self-administered questionnaire with measures simultaneously obtained with clinical and radiograph examinations. They found that self-reported history of root canal therapy had 90% sensitivity, 94% specificity, a positive predictive value of 86% and a negative predictive value of 95% among 58 adult patients to the Harvard School of Dental Medicine student dental clinic.[105] They conclude that self-reports of ET provide reasonably valid estimates of past root canal therapy (ET). A more recent study conducted by Gomes et al compared self-reported history of ET with ET status determined simultaneously from panoramic radiographs. They found that self-reported history of ET had 92% sensitivity, 89% specificity, 82% positive predictive value, and 95% negative predictive value.[106] The authors concluded that self-reported history of ET is a highly accurate method to predict historic ET.[106]

Endodontic Infection (EI)/Apical Periodontitis (AP) and CVD

The association between EI/AP and CVD has not been studied as extensively as periodontal disease and CVD. Previous studies have only considered the impact of EI/AP on CHD and have not yet considered the impact of EI/AP on other CVD outcomes. Additionally, the evidence linking EI/AP is inconsistent. The majority of previous studies

have found an association between EI/AP and increased risk of CHD[94, 101, 103, 107-110] while some studies failed to find an association between EI/AP and CHD[47, 111] and others were inconclusive.[102, 104, 112]

Three studies published in 2016 evaluated the association between EI/AP and CHD. Liljestrand et al used cross-sectional data from the 508 patients enrolled in the Finnish Parogene study and found that EI was independently associated with CHD (OR = 1.94) and with acute coronary syndrome (OR = 2.46).[101] Gomes et al used a retrospective cohort from the Baltimore Longitudinal Study of Aging and found that EI was independently associated with CHD (RR = 1.77).[102] Finally, An et al used a case-control study design and electronic medical record data and also found an independent association between AP and CHD (OR = 5.3).[103]

ARIC data have also been used to study the relationship between EI/AP and prevalent CHD. In 2009, Caplan et al conducted a cross-sectional study and found that among participants with 25 or more teeth, those reporting having had ET two or more times had 1.62 times higher odds of CHD compared with those reporting never having had ET.[94]

Despite the previous work devoted to understanding EI/AP and CHD, to our knowledge no studies have directly addressed the association between EI/AP and stroke, heart failure, or VTE. Additionally, most studies relied on cross-sectional or case-control study designs and prospective data are lacking. Studies of EI/AP and CVD published since 2014 are summarized in Table 2 below.

Table 2. Summary of studies on EI/AP and CVD since 2014

Study	Year	Study Design	Outcome	Association (95% CI)
Liljestrand et al[101]	2016	Cross-Sectional	CHD	OR=2.46 (1.09, 5.54)
Gomes et al[102]	2016	Retrospective Cohort	CVD	RR=1.77 (1.04, 3.02)
An et al[103]	2016	Cross-Sectional	CVD	OR=5.3(1.5, 18.4)
Petersen et al[108]	2014	Cross-Sectional	Aortic Atherosclerotic Burden	OR=1.61 (1.39,1.86)
Costa et al[109]	2014	Cross-Sectional	CHD	RR= 2.79 (1.1, 7.3)
Willershausen et al[110]	2014	Case-Control	MI	OR=1.54 1.10, 2.16)

Infection as a CVD Trigger

In addition to chronic and oral infections, infections have been studied as acute precipitants or triggers of CVD events. In their seminal paper, Smeeth et al conducted a large prospective study using data from the United Kingdom General Practice Research Database to investigate MI and stroke risk following acute infection. They found that risk of MI increased by a factor of 5 and stroke risk increased by a factor of 3 in the days following an acute upper respiratory infection, and to a lesser degree after a urinary tract infection. Risk gradually fell during the following weeks.[113]

Since then, numerous studies have sought to elucidate the triggering association between acute infections and CVD. In 2014, Dalager-Pedersen et al conducted a case-control study and determined that the risk of MI and stroke was greatly increased within 30 days of community-acquired bacteremia (RR = 17.70 compared to population controls, RR = 2.32 compared to hospitalized controls) but decreased within 6 months of infection.[114] Considering influenza infection and MI, Warren-Gash et al used electronic medical records and a case-crossover study design and found that MI risk was significantly raised during days 1-3 after acute respiratory infection (IRR = 4.19) with the effect tapering over time.[115] A 2012 study by Guan et al used antibody levels to detect past infection and found that the risk of MI was associated with the presence of IgG

antibodies to influenza virus A (OR = 5.5) and influenza virus B (OR = 20.3).[116] Another case-crossover study was done by Chew et al in 2012 and found an increased risk of acute coronary syndrome (OR = 7.5) 0-7 days following an infection compared to 7-8 weeks following an infection.[117] ARIC data were combined with the Cardiovascular Health Study (CHS) to study triggering by infection. Corrales-Medina et al found that CVD risk after pneumonia was highest in the first 30 days following infection (HR = 4.07) and decreased over time.[18]

Previous studies have also examined infection as a trigger of ischemic stroke. Dalager-Pedersen et al's 2014 study concluded that stroke risk is greatly increased within 30 days of community-acquired bacteremia (RR = 25.82 compared to population controls, RR = 2.41 compared to hospitalized controls) but decreases over time.[114] A study published in 2016 by Cowan et al used ARIC data and a case-crossover study design to show that within a population-based cohort, ischemic stroke risk is higher after hospitalization with infection.[118] Similar results where shown by Elkind et al using CHS data[119] and Fullerton et al using VIPS data.[120]

Infection as a trigger for VTE has received less research attention. A 2017 study by Cowan et al found an independent increase in VTE risk following a hospitalized infection (OR ~ 2.0) that decreased over time.[121] A previous study by Chen et al found an association between bacteremia and VTE (OR = 1.9).[122] An earlier study by Dalager-Pedersen et al found an association between pneumonia and both deep vein thrombosis (DVT) (HR = 1.78) and pulmonary embolism (PE) (HR = 1.97).[123] Rogers et al. used a case-crossover design to measure infection as a potential VTE trigger. They found that the adjusted incidence rate ratio for infection during the 90-day period before

hospitalization for VTE compared with infection during the control period was 2.90.[124] Studies of infection as a CVD trigger published since 2014 are summarized in Table 3 below.

Table 3. Summary of studies of infection as a CVD trigger since 2014

Study	Year	Study Design	Exposure	Outcome	Association (95% CI)
Dalager-Pedersen et al[114]	2014	Case-Control	Bacteremia	MI and Stroke	Population Controls MI RR = 20.86 (15.38, 28.29) Hospital Controls MI OR = 2.18 (1.80, 2.65)
Cowan et al[118]	2016	Case-Crossover	Inpatient Infections	Ischemic Stroke	14-day OR = 7.7 (2.1–27.3) 30-day OR = 5.7 (2.3–14.3 42-day OR = 4.5 (2.0–10.2) 90-day OR = 3.6 (2.1–6.5)
Fullerton et al[120]	2015	Case-Control	All Infections and Infection Vaccinations	Ischemic Stroke	Infection OR = 6.3 (3.2, 12.0) Poorly Vaccinated OR = 8.2 (2.5, 26.0)
Masrouha et al[123]	2016	Prospective Cohort	Pneumonia	DVT and PE	DVT – OR = 1.67 (1.32, 2.11) PE – OR = 2.18 (1.48, 3.22)
Chen et al[125]	2015	Retrospective Cohort	Pneumonia	DVT and PE	DVT – HR = 1.78 (1.39, 2.28) PE – HR = 1.97 (1.43, 2.72)
Dalager-Pedersen et al[122]	2014	Prospective Cohort	Community Acquired Bacteremia	VTE	OR = 1.9 (1.4, 2.7)

Chapter 2 – Mechanisms Linking Infection and CVD

Given the evidence of an association between both chronic and acute infections and CVD, substantial work has been done to elucidate the mechanisms linking infection and CVD. While a comprehensive review of the previous research is beyond the scope of this work, a summary of the relevant mechanisms and pathways is important.

Systemic infection refers to an infection that affects the entire body as microbes and their byproducts can gain systemic access via the circulatory system.[126] Evidence of infectious agents has been identified in studies of cardiovascular health. In 1999 Chiu identified multiple infectious agents in carotid endarectomy specimens and their corresponding atherosclerotic plaques.[127] Similarly, Haraszthy et al. studied carotid endarectomy specimens and detected the presence of periodontal pathogens in atherosclerotic plaques.[128]

Systemic infection and the subsequent immune system response induce a proinflammatory condition connected to multiple proposed mechanisms linking infection and cardiovascular disease.

First, vascular endothelial cells can be infected directly, initiating the inflammatory response inducing atherosclerosis. Infectious agents could accelerate or enhance the atherosclerotic process through recruitment and stimulation of proinflammatory cytokine proteins — such as IL-6, IL-1β, tumour necrosis factor, complement proteins, C-reactive protein (CRP), serum amyloid A-1 protein, coagulation proteins and fibrinogen and tissue growth factors in the arterial wall, as well as enhancement of lipid accumulation through stimulation of macrophage scavenger or LDL-receptors.[129] Damage to the vascular wall causes the expression of vascular cell adhesion molecule 1 and intercellular adhesion

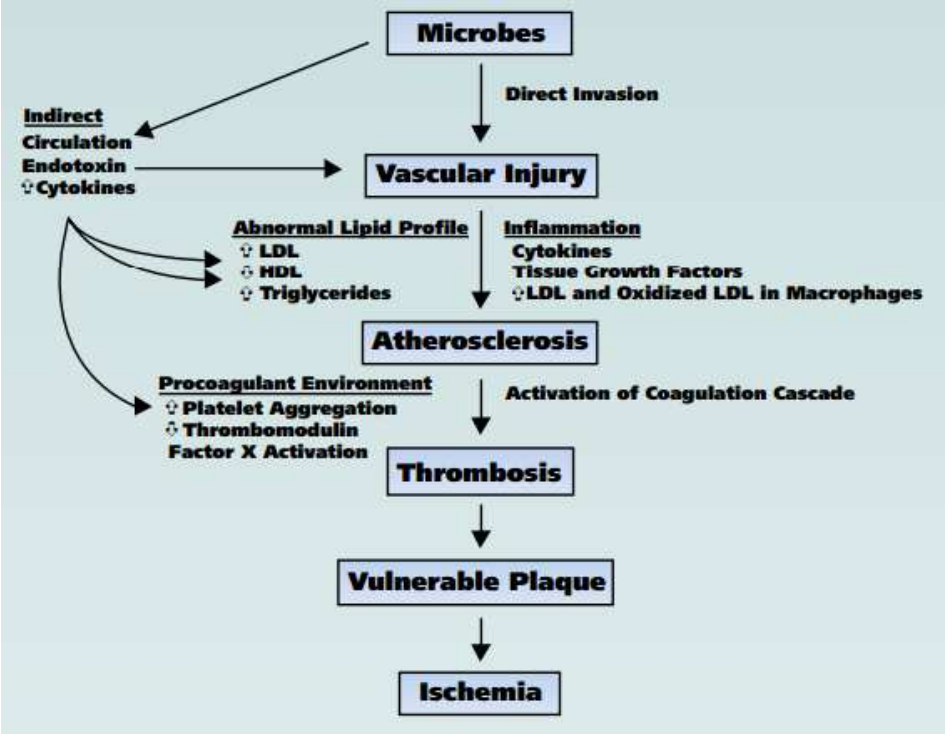
molecule 1 by endothelial cells, causing monocytes to bind to the endothelium, transmigrate into the vessel wall and differentiate into macrophages.[130] Macrophages contribute to the inflammatory cascade, leading to the recruitment of more macrophages into the vessel wall. Some macrophages also become lipid-laden foam cells, promote smooth muscle cell proliferation and migration, disorganization of the matrix membrane, and further endothelial cell dysfunction. Infectious agents acting directly on endothelial cells can induce thrombosis, and contribute to the formation and maturation of atherosclerotic plaques.

Infectious microbes could also indirectly influence the development and progression of atherosclerosis without directly invading the arterial endothelium. Release of endotoxin or lipopolysaccharide into the circulation could indirectly damage vascular endothelium or the immune response, and systemic cytokine release could result in lipid profile predisposing to atherosclerosis or could predispose the arterial environment to a procoagulant state.[129] Infection leads to the overexpression of tissue factor which activates factor X, an activator of thrombin. Activated factor X and thrombin induce coagulation and mediate the inflammatory response by acting on protease-activated receptors expressed on endothelial cells, platelets and leukocytes.[130] Other inflammatory cytokines impacted by infection, including IL-1β, IL-6, and TNF can also activate coagulation and inhibit fibrinolysis.

Figure 3 below, which summarizes the mechanisms linking infection and CVD, was taken from Fong et al.[129] It is also worth considering the epidemiologic evidence supporting the proposed mechanisms linking infection and CVD. Substantial evidence from previous studies has accumulated related to the relationship between infection and

systemic inflammation, atherosclerotic development, and platelet aggregation and hypercoagulability and the relationship between these mechanisms and CVD.[82]

Figure 3. Mechanisms linking infection and CVD



Inflammation

Infection impacts CVD through systemic inflammation. Multiple studies have found independent associations between infection and inflammatory markers. A meta-analysis conducted by Filardo et al found that CRP, IL-6, and fibrinogen levels were significantly higher in participants with pneumonia compared to participants without pneumonia.[131] Considering HIV infection and inflammatory markers, the meta-analysis by Vos et al found that HIV infection was associated with higher levels of C-reactive protein (CRP), interleukin-6 (IL-6) and d-dimer compared to uninfected persons.[132]

Existing studies have shown that periodontitis is associated with levels of systemic inflammatory markers including interleukin-6 (IL-6)[133], C-reactive protein (CRP)[133, 134] and soluble intercellular adhesion molecule-1 (sICAM-1).[135] A meta-analysis performed

by Freitas et al found that periodontal treatment was independently associated with higher CRP levels.[136] Similarly, a meta-analysis conducted in 2013 by Gomes et al found that AP was independently associated with increased levels of CRP, IL-1, IL-2, IL-6, asymmetrical dimethylarginine, IgA, IgG, and IgM.[137]

Previous work on the association between inflammatory markers and CVD has been extensive. A number of previous studies have found associations between inflammatory markers and CVD. In their seminal paper and meta-analysis, Danesh et al found that fibrinogen, CRP, albumin, and leukocyte count were all independently associated with CHD.[138] More recent meta-analyses have found additional inflammatory markers that are associated with CVD and expanded understanding of inflammation as a CVD risk factor.[139-141] An association between inflammatory markers and VTE has also been established.[142] Inflammatory markers have also been shown to be predictive of future cardiovascular risk.[143, 144]

Atherosclerotic Development

Multiple studies have identified an association between infection and atherosclerotic development.[145-147] A meta-analysis performed by Huang et al found an independent association between hepatitis C infection and carotid atherosclerosis.[148] Periodontal disease is also independently associated with atherosclerotic development. Zeng et al recently performed a meta-analysis and found an independent association between periodontal disease and carotid atherosclerosis.[149] Earlier meta-analyses found positive associations between periodontal treatment and makers of endothelial function and atherosclerosis.[150, 151]

Platelet Aggregation/Hypercoagulability

Infection can influence CVD by increasing platelet aggregation and hypercoagulability. Liang et al found that persons infected with HIV had increased monocyte platelet aggregates compared to uninfected persons.[152] Perumal et al found an association between platelet activation and chronic periodontitis.[153] Similarly, Banthia et al found that periodontal therapy was associated with a decrease in coagulant markers including leukocyte count, differential leukocyte count, and platelet count.[154] Roth et al studied human aortic endothelial cells and found that P. gingivalis is associated with markers of both coagulation and thrombosis.[155] Animal models have also found associations between infection and platelet aggregation and hypercoagulation.[156, 157]

Infections that gain access to the entire body through the circulatory system may impact CVD through systemic inflammation that leads to platelet aggregation and hypercoagulability and atherosclerotic development.

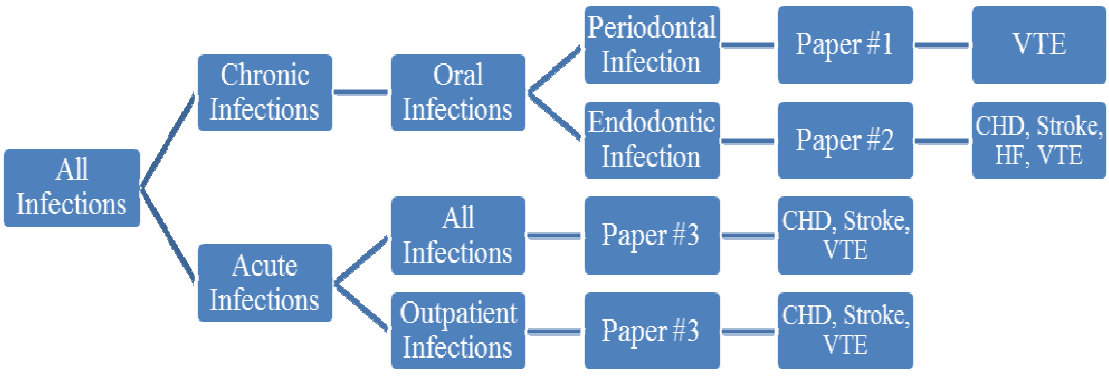
Summary of Background and Rationale

While the existing evidence linking periodontal disease and CHD and stroke is promising, a potential association between periodontal disease and VTE has received less research attention and requires further study. Likewise, the relationship between EI/AP and CHD has not been studied extensively and the inconsistent findings of previous studies suggest the need for further research to clarify the relationship. To our knowledge no studies have directly addressed the association between EI/AP and stroke, heart failure, or VTE. Further research is needed to identify potential relationships between EI/AP and these outcomes. Finally, previous studies on infection as a CVD trigger often only included hospitalized infections as their exposure of interest. Further, the magnitude

and duration of increased cardiovascular risk has varied greatly between studies and remains under debate. Additional research is warranted to elucidate the magnitude and duration of risk associated with infections across all CVD outcomes.

We propose to use longitudinal data from the ARIC study, the Longitudinal Investigation of Thromboembolism Etiology (LITE) ancillary study, the Dental-ARIC (D-ARIC) ancillary study, and the corresponding ARIC study participant Centers for Medicare and Medicaid Services (CMS) data to examine the relationship between periodontal disease and VTE, endodontic therapy and CVD, and infection as a trigger for CVD events. We hypothesize that periodontal disease is independently associated with VTE, endodontic therapy is independently associated with risk of incident CVD, and infection is a trigger of CVD events. The ARIC study is well-positioned to extend the body of research in this area because of the large number of validated CVD events, objective periodontal data, information on both inpatient and outpatient infections, and extensive data collection on potential confounders.

Figure 4. Summary of dissertation papers



Chapter 3 – Study Design and Data Collection

Atherosclerosis Risk in Communities (ARIC) Study

The ARIC study is a multi-center population-based prospective cohort study designed to investigate the etiology and natural history of atherosclerosis in middle-aged Americans.[158] At baseline in 1987-1989 (visit 1), 15,792 white and black men and women were selected from 4 US communities: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland.[158] Subsequent exams took place during 1990 to 1992 (visit 2), 1993 to 1995 (visit 3), 1996 to 1998 (visit 4), and 2011 to 2013 (visit 5). Visit 6 (2016-2017) is currently underway.

Extensive information on CVD risk factors and outcomes was collected at each exam. Additionally, annual telephone questionnaires were administered to update information on CVD events and hospitalizations; phone calls have occurred semiannually since 2012. All hospitalizations and deaths in ARIC participants occurring through December 31, 2013 were identified by annual telephone follow-up call, review of hospital discharge lists, and death certificates. Data were abstracted from hospital and death records, next-of-kin interviews, and physician-completed questionnaires. CVD events were classified by a combination of computer algorithm and adjudicated physician review; disagreements were adjudicated by the ARIC Mortality and Morbidity Classification Committee using standardized ARIC criteria.[159] A detailed discussion of the ARIC study design, methods, and objectives is provided elsewhere.[158]

Longitudinal Investigation of Thromboembolism Etiology (LITE)

LITE is a prospective study of VTE occurrence in 2 pooled, multicenter, longitudinal population-based cohort studies: the Atherosclerosis Risk in Communities

(ARIC) study and the Cardiovascular Health Study (CHS).[160] All possible VTE cases among ARIC participants occurring through December 31, 2011 were identified using hospital discharge codes from ARIC study participant hospitalizations. Hospital records were independently reviewed by two study physicians to determine the VTE case status. Differences were adjudicated by physician discussion. A detailed discussion of the LITE study design, methods, and objectives is provided elsewhere.[160]

Dental ARIC (D-ARIC)

The purpose of D-ARIC was to determine the prevalence, extent, and severity of periodontal conditions in the dentate ARIC population and describe the associations between those conditions and prevalent CHD and atherosclerosis.[48] The D-ARIC study was an ARIC ancillary study that took place among ARIC participants at all 4 study sites during visit 4 (1996-1998). All ARIC participants were screened for participation over the phone when they were contacted to schedule visit 4. Participants who had no natural teeth were not eligible to participate in D-ARIC. Additionally, participants who had medical contraindications to periodontal probing or who required antibiotic prophylaxis for periodontal probing were also excluded from the dental examination.

D-ARIC consisted of a dental questionnaire and a dental examination adapted from the protocol used for the Third National Health and Nutrition Examination Survey (NHANES III).[161] Examination components included complete soft tissue screening, caries status and plaque score, collection and storage of crevicular fluid, and microbial sampling and analysis of plaque samples. Assessments related to periodontal disease included the gingival index to assess inflammation, probing pocket depth around each tooth, cemonto-enamel junction (CEJ) to assess the distance from the CEJ to the gingival

margin (gingival recession), and bleeding on probing for each tooth. Attachment level was calculated from the sum of pocket depth and gingival recession scores.

Dental examiners were thoroughly trained using standardized procedures to ensure that measurements were both valid and reliable. Further, the dental examiners were calibrated against a standard examiner, as well as each other to ensure consistency across examiners and sites. Details of the D-ARIC study objectives and methodology have been described in a previous publication.[162]

Centers for Medicare and Medicaid Services (CMS)

Medicare is a national social health insurance program, administered by the US federal government since 1966.[163] Medicare coverage is available to the elderly (≥65), those with disability, those with end stage renal disease, and those with amyotrophic lateral sclerosis. The CMS Medicare data are healthcare encounter reimbursable claims data for individuals who are eligible for and enrolled in CMS Medicare services. The ARIC study has an interagency agreement with the CMS to obtain Medicare data for ARIC cohort participants. CMS claims for inpatient and outpatient services are available for eligible ARIC participants from 1991-2013. ARIC participants are matched to Medicare claims data based on social security number, sex, and date of birth.[164] Of the 14,899 ARIC study participants Medicare eligible in 1991, 98.7% were successfully matched with their Medicare beneficiary summary file. Matched participants are linked to inpatient, outpatient, and carrier files.

Claims data are available for inpatient services covered under Medicare Part A contained in the inpatient claim file which contains final action claims data submitted by inpatient hospital providers for reimbursement of facility costs. Also, the MedPAR file is

constructed from inpatient claims and contains inpatient hospital and skilled nursing facility (SNF) final action stay records which summarizes all services rendered to a beneficiary from the time of admission to a facility through discharge. The outpatient claims file contains final action claims data submitted by institutional outpatient providers for services covered under Medicare Part B. The carrier claim file contains final action claims data submitted by non-institutional providers including clinicians, independent clinical laboratories, ambulance providers, and free-standing ambulatory surgical centers.

Medicare data have a number of limitations. First, data are unavailable for ARIC participants who are ineligible for Medicare coverage. Medical claims data are, for the most part, not available for beneficiaries enrolled in managed care (HMO) organizations, such as Medicare Advantage insurance plans. These plans are not required to submit claims data for beneficiaries thus CMS files will have incomplete claims data for such participants.

Chapter 4 – Manuscript #1: Periodontal Disease and Incident Venous Thromboembolism: The Atherosclerosis Risk in Communities Cohort

Abstract

Background

Periodontal disease has been identified as a cardiovascular disease (CVD) risk factor but few studies have considered the relationship between periodontal disease and venous thromboembolism (VTE). Periodontal disease may be related to VTE through infection-induced inflammation that contributes to platelet aggregation and hypercoagulability. We hypothesized that periodontal disease is independently associated with incident VTE risk.

Methods

We used a prospective cohort study design using longitudinal data from the Atherosclerosis Risk in Communities (ARIC) study, the Longitudinal Investigation of Thromboembolism Etiology (LITE) ancillary study, and the Dental-ARIC (D-ARIC) ancillary study to study the relationship between periodontal disease and incident VTE. Periodontal disease was determined using self-reported tooth loss due to gum disease and two clinical periodontal disease classifications. Cox-proportional hazards regression models were used to estimate hazard ratios and 95% confidence intervals across self-reported tooth loss due to gum disease statuses and clinical periodontal disease classifications. Adjusted models were constructed to include relevant confounders including demographic and SES factors, VTE risk factors, and dental hygiene and access to care variables.

Results

Self-reported periodontal disease was associated with 30% higher VTE risk that remained significant or borderline significant after adjustment. Crude associations

between clinical periodontal disease classifications were attenuated with adjustment and were no longer significant.

Conclusion

Further research is needed to determine if periodontal disease is independently associated with VTE risk and if periodontal prevention and treatment could reduce VTE risk.

Introduction

Periodontal disease is a chronic inflammatory disease caused by bacterial infection of the supporting tissues around the teeth.[29] Periodontitis is common with 46% of US adults having any periodontitis and 8.9% having severe periodontitis.[32] It is a significant contributor to tooth loss among adults in the United States.[34] The primary mechanism linking periodontitis and venous thromboembolism (VTE) is through infection-induced systemic inflammation that contributes to platelet aggregation and hypercoagulability.[82] Existing studies have shown that periodontitis is associated with higher levels of systemic inflammatory markers including interleukin-6 (IL-6)[133], C-reactive protein (CRP)[133, 134], and soluble intercellular adhesion molecule-1 (sICAM-1).[135] Higher levels of these inflammatory markers have also been associated with increased risk of cardiovascular disease (CVD) and VTE.[134, 142, 165, 166] The potential pathway linking periodontal infection and VTE risk is presented in the causal diagram in Figure 5 below.

VTE, comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common, life-threatening disease in the United States with over 500,000 hospitalized cases annually.[167] The Longitudinal Investigation of Thromboembolism Etiology (LITE) study found an incidence rate of 1.92 per 1000 person years for VTE in a community-based cohort of middle and older aged individuals and a 28-day case fatality rate of 11%.[168]

Previous epidemiologic research has identified VTE risk factors including immobilization, surgery, trauma, cancer, older age, family history of VTE, genetics, oral contraceptive use, and obesity.[169, 170] Though multiple individual studies and meta-analyses have reported an association between periodontal infection and increased risk of

CVD,[82, 83] to date only one study has explored a possible association between periodontitis and VTE. In that study, Sanchez-Siles et al. found that periodontitis was more prevalent among 97 VTE patients than in the 100 healthy controls ($p<.001$), thus supporting an association between periodontal disease and VTE risk.[93]

The prospective ARIC cohort has ancillary studies which conducted dental exams and validated VTE events. Using these data we examined the relationship between periodontal disease and risk of incident VTE. We hypothesized that periodontal disease is independently associated with incident VTE risk and that the risk is graded such that the association between periodontal disease and VTE is highest among those with more severe periodontal disease.

Methods

LITE a prospective study of VTE occurrence in 2 pooled, multicenter, longitudinal population-based cohort studies: the Atherosclerosis Risk in Communities (ARIC) study and the Cardiovascular Health Study (CHS). The LITE study design, methods, and VTE incidence rates have been described in detail elsewhere.[160] For these analyses, CHS data were excluded and only ARIC data were included due to the presence of dental information collected at visit 4 during the dental ancillary study (D-ARIC). The ARIC study is a multi-center population-based prospective cohort study designed to investigate the etiology and natural history of atherosclerosis in middle-aged Americans.[158] At baseline in 1987-1989 (visit 1), 15,792 white and black men and women were selected from 4 US communities: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland.[158] Subsequent exams took place during 1990 to 1992 (visit 2), 1993 to 1995 (visit 3), 1996

to 1998 (visit 4), 2011 to 2013 (visit 5), and 2016-2017 (visit 6 currently underway). A detailed discussion of the ARIC study design, methods, and objectives is provided elsewhere.[158]

The purpose of D-ARIC was to determine the prevalence, extent, and severity of periodontal conditions in the dentate ARIC population and describe the association between those conditions and prevalent CHD and atherosclerosis.[48] The D-ARIC study was an ARIC ancillary study that took place among ARIC participants at all study sites during visit 4 (1996-1998). All ARIC participants who had natural teeth and who had no contraindications to or required antibiotic prophylaxis for periodontal probing were eligible to participate in D-ARIC. Details of the D-ARIC study objectives and methodology have been described in a previous publication.[162]

For the present analyses, we used a prospective cohort study design. Visit 4 (1996-1998) which was attended by 11,656 participants was used as the baseline for these analyses. All ARIC participants who reported being edentulous at exam 4 (n=1,651) and those who completed the D-ARIC exam (n=6,793) were included in these analyses. Those of races other than black and white (27), those with prevalent VTE at baseline (218) as determined by self-report at study entry or adjudicated VTE events occurring prior to baseline (visit 4), and those taking anticoagulants at baseline (visit 4) (80) were excluded from these analyses leaving us with a final sample size of n=8,119.

Periodontal Disease Ascertainment

The exposure of interest is periodontitis and was determined using self-reported tooth loss due to gum disease and two clinical periodontal disease classifications. Self-reported tooth loss due to gum disease was based on yes/no responses to the question

"Did you lose any teeth because of gum disease". Clinical periodontitis was assessed using clinical measures collected during the D-ARIC oral examination including probing pocket depth and gingival recession on 6 sites for all teeth. Attachment level was calculated from the sum of pocket depth and gingival recession scores. The first clinical case definition used was the CDC Periodontal Disease Surveillance workgroup in collaboration with the American Academy of Periodontology (CDC/AAP) definition.[39] This 4-level definition is as follows:

No periodontitis: Not meeting the definition of mild, moderate, or severe periodontitis

Mild periodontitis: ≥2 interproximal (between teeth) sites with attachment loss ≥3mm, and ≥2 interproximal sites with probing depth ≥4 mm (not on same tooth) or one site with probing depth ≥5 mm

Moderate periodontitis: ≥2 interproximal sites with attachment loss ≥4 mm (not on same tooth), or ≥2 interproximal sites with probing depth ≥5 mm (not on same tooth)

Severe periodontitis: ≥ 2 interproximal sites with attachment loss ≥6 mm (not on same tooth) and ≥1 interproximal site with probing depth ≥5 mm

The second clinical periodontal disease classification used was the Periodontal Profile Class (PPC) proposed by Morelli et al.[40] This uses 7 tooth-level clinical parameters (interproximal attachment level, probing depth, bleeding on probing, gingival inflammation index, plaque index, the presence/absence of full prosthetic crowns for each tooth, and tooth status presence) to identify seven distinct periodontal profile classes (PPC).

This 7-level definition is as follows:

PPC-A: Health

PPC-B: Mild Disease

PPC-C: High Gingival Inflammation Index

PPC-D: Tooth Loss

PPC-E: Posterior Disease

PPC-F: Severe tooth Loss

PPC-G: Severe Disease

Covariate Ascertainment

Demographic variables including age at visit 4, sex, race, and study center were assessed at baseline (visit 4) or updated from information collected at study enrollment (visit 1). Education (some high school or less, high school diploma, college degree or higher) was assessed at study entry (visit 1) while income (<$25,000/year, $25,000 – <$50,000/year, $50,000 – <$75,000/year, >$75,000/year) was assessed at baseline (visit 4). Common risk factors including smoking (current, former, never), alcohol consumption (grams/week), BMI (Kg/M^2), and statin use (yes/no) were assessed at baseline (visit 4). Finally, oral hygiene and access to care variables including medical insurance status (private insurance, Medicare/Medicaid only, none), last dental visit (<6 months ago, 6 months – <2 years ago, 2 – <5 years ago, >5 years ago), dental visit frequency (regularly, only for discomfort or repair, don't regularly visit the dentist), and having a current dentist (yes/no) were collected during the dental history questionnaire administered at baseline (visit 4). Baseline (visit 4) C-reactive protein (CRP) levels were measured in 2008 on plasma frozen at −70°C from visit 4 by the immunoturbidimetric

assay using the Siemens (Dade Behring) BNII analyzer (Dade Behring, Deerfield, Ill), performed according to the manufacturer's protocol.

Outcome Ascertainment

The outcome of interest was incident VTE. VTE events were defined as all PEs and DVTs occurring in the legs (n=755). Potential incident VTE events were identified using diagnosis codes (415.1x, 451, 451.1x, 451.2, 451.8x, 451.9, 453, 453.0, 453.1, 453.2, 453.8, 453.9, 996.7x, 997.2, 999.2, 38.7), hospital records, physician and consultant reports, and discharge summaries according to LITE study protocol.[168] Hospital records were independently reviewed and adjudicated by two study physicians to determine the VTE case status. Differences were resolved by physician discussion.[168] The hospital admission date abstracted from the patient medical record was considered the VTE date.

Statistical Analysis

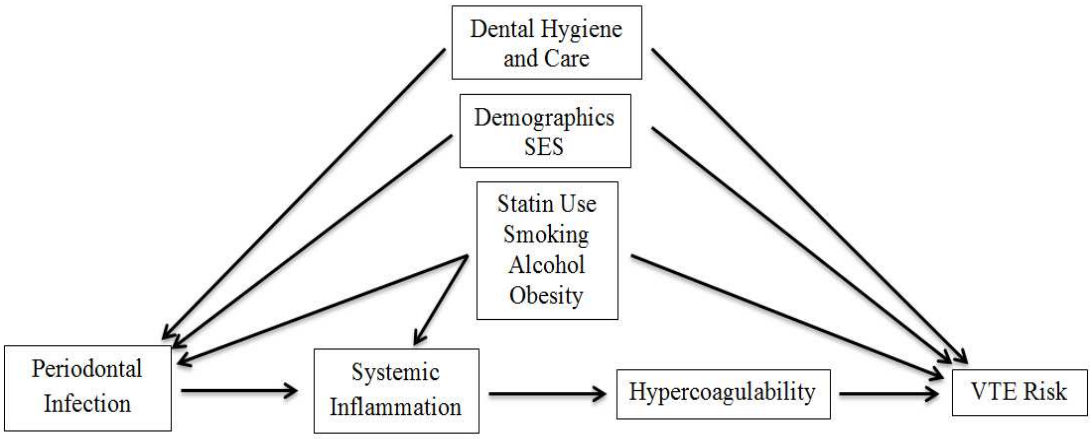
Descriptive statistics were calculated for study participant characteristics at baseline (visit 4) by periodontal disease category. Baseline (visit 4) C-reactive protein (CRP) levels were compared using linear regression controlling for age, race/center, and sex. Means were compared using the least squares means procedure in SAS 9.3. Associations between self-reported periodontal disease status and clinical periodontal disease classifications were assessed using cross tabulations and the chi-squared test. Cox-proportional hazards regression models were used to estimate hazard ratios and 95% confidence intervals across self-reported tooth loss due to gum disease statuses and clinical periodontal disease classifications (CDC/AAP and PPC). Those who report being edentulous and those with different levels of periodontal disease were compared against those without periodontal disease (referent). The proportional hazards assumption was

assessed by visual inspection of the Kaplan-Meier (KM) curves and by testing the interaction between periodontal disease status and follow-up time.

Crude (unadjusted) models and those adjusting for potential confounders were constructed. Covariate values were taken from assessments performed at the ARIC visit 4 exam. Only risk factors common to both periodontal disease and VTE were considered. Variable selection and model construction was informed by the causal diagram presented in Figure 5 below. In addition to crude models, model 1 included demographic and SES variables including age, sex, race/center, education, and income. Model 2 added adjustment for relevant periodontal risk factors that are also associated with VTE including smoking,[170, 171] alcohol consumption,[172, 173] BMI,[174, 175] and statin use.[176, 177] Finally, model 3 additionally included adjustment for variables related to oral hygiene and access to care including medical insurance status, last dental visit, dental visit frequency, and having a current dentist.

Finally, we performed a sensitivity analysis in which the association between periodontal disease and VTE was considered excluding participants who reported a history of endodontic therapy since endodontic infection may trigger a similar inflammatory response as periodontal infection.

Figure 5. Causal diagram of pathways linking periodontal infection and venous thromboembolism risk



Follow- up time began at entry into the study (visit 4) and extended to the first outcome, dropping out of the study, death, or else, December 31, 2011.

Power and Sample Size

Since we used previously collected observational data, the sample size for these analyses was fixed. With a fixed sample size, the question of interest becomes what is the minimum detectable effect (MDE) of periodontal disease on VTE for our study. We focused the MDE analysis on the primary analysis using the CDC/AAP periodontal disease definition. Using this definition, exposure was categorized as no periodontitis, mild periodontitis, moderate periodontitis, severe periodontitis, and edentulous. Frequency of each exposure classification is found in Table 4 below.

Table 4. Frequency of CDC/AAP periodontal disease classifications among ARIC participants at baseline (visit 4 – 1996-1998)

Periodontal Disease	N
No Periodontitis	750
Mild Periodontitis	1,976
Moderate Periodontitis	2,708
Severe Periodontitis	1,140
Edentulous	1,545

Since each exposure classification was compared to those without periodontal disease, we focused our MDE analysis on comparing those with severe periodontitis to those without periodontitis since those with severe periodontitis make up the smallest exposure classifications and thus the lowest powered comparison with the largest MDE. Additionally, we also conducted a MDE analysis comparing edentulous participants to those with no periodontitis since they represented the most extreme exposure categories.

The MDE analyses were performed using the Power and Sample Size Program.[178, 179] Our study had 1,140 participants with severe periodontitis and 1,545 participants who were edentulous compared to 750 participants without periodontitis approximately 6% of whom will experience a VTE event during follow-up. Based on these criteria, Table 5 below contains MDE HRs for α-level (type I error rate) equal to 0.05 across levels of power (1-β).

Table 5. Minimum detectable effect hazard ratios by power levels

	Power = 80%	Power = 85%	Power = 90%
Severe Periodontitis	1.56	1.60	1.66
Edentulous	1.59	1.64	1.70

By convention, we used 80% power giving us a MDE = HR = 1.56 and 1.59 for those with severe periodontitis and those who are edentulous compared to those with no periodontitis respectively. This effect size is consistent with previous studies[51, 81] and suggests that the anticipated and meaningful effect size is detectable by our study.

Results

Baseline (ARIC visit 4) characteristics are provided in Tables 6, 7, and 8 for study participants by self-reported periodontal disease status, CDC/AAP clinical periodontal disease status, and PPC periodontal disease classification respectively. Across exposure classifications, periodontal disease was more common among males compared to females

and among blacks compared to whites. Periodontal disease was more common among those of low SES status compared to those of high SES status. Those with periodontal disease were more likely to be smokers compared to those without periodontal disease. Finally, those with periodontal disease were less likely to have medical insurance, a current dentist, and visit the dentist regularly.

Table 6. Baseline (Visit 4 – 1996-1998) characteristics of ARIC participants by self-reported periodontal disease status

	Tooth Loss from Gum Disease	No Tooth Loss from Gum Disease
Total, count (%)	1,098 (13.9)	6,784 (86.1)
Age (years), mean ± SD	63.5 (5.7)	62.6 (5.6)
Sex, count (%)		
Male	492 (44.8)	3,061 (45.1)
Female	606 (55.2)	3,723 (54.9)
Race, count (%)		
White	725 (66.0)	5,484 (80.8)
Black	373 (34.0)	1,300 (19.2)
Education, count (%)		
Some High School	332 (30.3)	1,169 (17.3)
High School Diploma	440 (40.2)	2,911 (43.0)
College Degree	323 (29.5)	2,694 (37.8)
Income, count (%)		
<$24,999	462 (43.5)	1,846 (27.7)
$25,000-$50,000	357 (33.6)	2,352 (35.3)
$50,000-$74,999	141 (13.3)	1,263 (19.0)
>$75,000	83 (7.8)	1,050 (15.8)
Refused	20 (1.9)	148 (2.2)
Smoker, count (%)		
Current	246 (22.6)	916 (13.6)
Former	510 (46.8)	2,887 (42.8)
Never	333 (30.6)	2,960 (43.7)
Statin Use, count (%)		
No	934 (85.3)	6,077 (89.8)
Yes	161 (14.7)	688 (10.2)
BMI (Kg/M^2), mean ± SD	29.3 (5.7)	28.7 (5.5)
Alcohol (g/week), mean ± SD	36.5 (86.5)	32.8 (77.8)
Medical Care Payment, count (%)		
Health Plan	839 (77.0)	5,919 (87.6)
Medicare/Medicaid Only	162 (14.9)	488 (7.2)
None	89 (8.2)	350 (5.2)
Current Dentist, count (%)		
Yes	762 (69.9)	5,654 (83.6)
No	328 (30.1)	1,113 (16.5)
Last Dental Visit, count (%)		
Within last 6 months	430 (39.5)	3,721 (54.9)
6 months to 2 years ago	200 (18.4)	1,517 (22.4)
2 to 5 years ago	173 (15.9)	664 (9.8)
More than 5 years ago	287 (26.3)	871 (12.9)
When do you visit Dentist, count (%)		
Regular Basis	438 (40.0)	4,375 (64.5)
Discomfort/Something Fixed	568 (51.9)	2,126 (31.4)
Don't go to Dentist	78 (7.1)	217 (3.2)
Other	10 (0.9)	61 (0.9)

Table 7. Baseline (Visit 4 – 1996-1998) characteristics of ARIC participants by CDC/AAP periodontal disease classification

	Healthy	Mild	Moderate	Severe	Edentulous
Total, count (%)	750 (9.2)	1,976 (24.3)	2,708 (33.4)	1,140 (14.0)	1,545 (19.0)
Age (years), mean ± SD	61.9 (5.5)	61.6 (5.6)	62.8 (5.6)	63.0 (5.6)	64.2 (5.6)
Sex, count (%)					
Male	203 (27.1)	713 (36.1)	1372 (50.7)	728 (63.9)	652 (42.2)
Female	547 (72.9)	1263 (63.9)	1336 (49.3)	412 (36.1)	893 (57.8)
Race, count (%)					
White	633 (84.4)	1541 (78.0)	2273 (83.9)	873 (76.6)	1024 (66.3)
Black	119 (15.6)	435 (22.0)	435 (16.1)	267 (23.4)	521 (33.7)
Education, count (%)					
Some High School	88 (11.8)	199 (10.1)	375 (13.9)	219 (19.2)	697 (45.2)
High School Diploma	368 (49.1)	798 (40.5)	1177 (43.5)	487 (42.7)	610 (39.6)
College Degree	293 (39.1)	975 (49.4)	1152 (42.6)	434 (38.1)	234 (15.2)
Income, count (%)					
<$24,999	192 (25.9)	427 (22.0)	617 (23.1)	329 (29.3)	839 (57.0)
$25,000-$50,000	275 (37.1)	629 (32.4)	1015 (38.0)	421 (37.5)	439 (29.8)
$50,000-$74,999	130 (17.5)	442 (22.8)	538 (20.2)	208 (18.5)	120 (8.2)
>$75,000	128 (17.3)	397 (20.5)	434 (16.3)	147 (13.1)	43 (2.9)
Refused	16 (2.2)	45 (2.3)	64 (2.4)	19 (1.7)	31 (2.1)
Smoker, count (%)					
Current	54 (7.2)	158 (8.0)	351 (13.0)	257 (22.7)	396 (26.0)
Former	264 (35.3)	774 (39.3)	1264 (46.9)	551 (48.6)	636 (41.7)
Never	431 (57.5)	1038 (52.7)	1080 (40.1)	326 (28.8)	494 (32.4)
Statin Use, count (%)					
No	671 (89.8)	1,793 (91.0)	2,430 (90.0)	998 (87.7)	1,329 (86.5)
Yes	76 (10.2)	178 (9.0)	270 (10.0)	140 (12.3)	208 (13.5)
BMI (Kg/M^2), mean ± SD	28.6 (5.8)	28.3 (5.3)	28.7 (5.2)	28.8 (5.4)	29.6 (6.2)
Alcohol (g/week), mean ± SD	21.3 (50.5)	28.9 (66.5)	37.9 (81.2)	46.8 (95.8)	25.1 (84.6)
Care Payment, count (%)					
Health Plan	679 (90.7)	1771 (89.9)	2434 (90.2)	982 (86.6)	1058 (69.3)
Medicare/Medicaid Only	41 (5.5)	104 (5.3)	144 (5.3)	82 (7.2)	321 (21.0)
None	29 (3.9)	95 (4.8)	122 (4.5)	70 (6.2)	147 (9.6)
Current Dentist, count (%)					
Yes	692 (92.6)	1811 (92.4)	2398 (89.0)	934 (82.4)	696 (46.7)
No	55 (7.4)	149 (7.6)	298 (11.1)	199 (17.6)	795 (53.3)
Last Dental Visit, count (%)					
Within last 6 months	472 (63.3)	1272 (64.7)	1674 (62.2)	661 (58.4)	138 (9.2)
6 months to 2 years ago	171 (22.9)	478 (24.3)	649 (24.1)	238 (21.0)	216 (14.4)
2 to 5 years ago	67 (9.0)	136 (6.9)	215 (8.0)	199 (10.5)	325 (21.7)
More than 5 years ago	36 (4.8)	80 (4.1)	153 (5.7)	113 (10.0)	817 (54.6)
Dentist Visit, count (%)					
Regular Basis	556 (74.5)	1539 (78.3)	1988 (73.7)	707 (62.4)	91 (6.0)
Discomfort/Repair	175 (23.5)	396 (20.2)	647 (24.0)	392 (34.6)	1172 (78.0)
Don't go to Dentist	5 (0.7)	19 (1.0)	36 (1.3)	19 (1.7)	227 (15.1)
Other	10 (1.3)	11 (0.6)	26 (1.0)	16 (1.3)	12 (0.8)

Table 8. Baseline (Visit 4 – 1996-1998) characteristics of ARIC participants by periodontal profile class

	A	B	C	D	E	F	G	Edentulous
Total, count (%)	1,803 (22.2)	1,012 (12.5)	674 (8.3)	769 (9.5)	965 (11.9)	866 (10.7)	785 (6.0)	1,545 (19.0)
Age (years), mean ± SD	61.7 (5.5)	62.3 (5.8)	61.5 (5.5)	63.6 (5.6)	62.8 (5.6)	62.9 (5.5)	61.7 (5.8)	64.2 (5.6)
Sex, count (%)								
Male	609 (33.8)	542 (53.6)	300 (44.5)	347 (45.1)	535 (55.4)	378 (43.7)	305 (62.9)	652 (42.2)
Female	1,194 (66.2)	470 (46.4)	374 (55.5)	422 (54.9)	430 (44.6)	488 (56.4)	180 (37.1)	893 (57.8)
Race, count (%)								
White	1,742 (96.6)	980 (96.8)	188 (27.9)	629 (81.8)	948 (98.2)	570 (65.8)	263 (54.2)	1,024 (66.3)
Black	61 (3.4)	32 (3.2)	486 (72.1)	140 (18.2)	17 (1.8)	296 (34.2)	222 (45.8)	521 (33.7)
Education, count (%)								
Some High School	97 (5.4)	84 (8.3)	157 (23.4)	126 (16.4)	57 (5.9)	248 (28.6)	112 (23.1)	697 (45.2)
High School Diploma	755 (41.9)	454 (45.0)	216 (32.2)	375 (48.8)	431 (44.7)	408 (47.1)	191 (39.4)	610 (39.6)
College Degree	948 (52.7)	472 (46.7)	298 (44.4)	268 (34.9)	476 (49.4)	210 (24.3)	182 (37.5)	234 (15.2)
Income, count (%)								
<$24,999	252 (14.1)	168 (16.8)	269 (41.9)	196 (26.0)	141 (14.7)	369 (43.3)	170 (36.1)	839 (57.0)
$25,000-$50,000	606 (33.8)	364 (36.4)	210 (32.7)	326 (43.2)	361 (37.6)	319 (37.4)	154 (32.7)	439 (29.8)
$50,000-$74,999	444 (24.8)	231 (23.1)	101 (15.7)	141 (18.7)	228 (23.7)	97 (11.4)	76 (16.1)	120 (8.2)
>$75,000	441 (24.6)	215 (21.5)	51 (7.9)	77 (10.2)	214 (22.3)	52 (6.1)	56 (11.9)	43 (2.9)
Refused	49 (2.7)	23 (2.3)	11 (1.7)	14 (1.86)	17 (1.8)	15 (1.8)	15 (3.2)	31 (2.1)
Smoker, count (%)								
Current	123 (6.8)	64 (6.3)	85 (12.8)	125 (16.3)	161 (16.7)	187 (21.7)	75 (15.6)	396 (26.0)
Former	742 (41.2)	418 (41.4)	267 (40.2)	346 (45.2)	495 (51.3)	388 (45.1)	197 (41.0)	636 (41.7)
Never	936 (52.0)	528 (52.3)	312 (47.0)	295 (38.5)	309 (32.0)	286 (33.2)	209 (43.5)	494 (32.4)
Statin Use, count (%)								
No	1,624 (90.1)	901 (89.1)	611 (91.5)	683 (89.1)	862 (89.3)	768 (89.4)	443 (91.5)	1,329 (86.5)
Yes	178 (9.9)	110 (10.9)	57 (8.5)	84 (11.0)	103 (10.7)	91 (1036)	41 (8.5)	208 (13.5)
BMI (Kg/M^2), mean ± SD	27.4 (4.8)	28.3 (4.9)	30.3 (6.2)	28.9 (5.1)	28.2 (5.1)	29.6 (6.0)	29.5 (5.9)	29.6 (5.9)
Alcohol (g/week), mean ± SD	35.1 (71.9)	36.4 (75.9)	22.0 (72.9)	32.4 (77.6)	52.7 (94.4)	27.6 (70.3)	29.5 (73.5)	25.1 (84.6)
Care Payment, count (%)								
Health Plan	1,727 (95.8)	946 (93.6)	508 (76.4)	989 (89.8)	923 (95.7)	693 (80.4)	380 (79.0)	1,058 (69.3)
Medicare/Medicaid Only	42 (2.3)	30 (3.0)	84 (12.6)	44 (5.7)	20 (2.1)	92 (10.7)	59 (12.3)	321 (21.0)
None	33 (1.8)	35 (3.5)	73 (11.0)	34 (4.4)	22 (2.3)	77 (8.9)	42 (8.7)	147 (9.6)
Current Dentist, count (%)								
Yes	1,750 (97.8)	949 (94.2)	506 (75.5)	706 (92.2)	928 (96.5)	657 (76.5)	339 (70.3)	696 (46.7)
No	39 (2.2)	59 (5.9)	164 (24.5)	60 (7.8)	34 (3.5)	202 (23.5)	143 (29.7)	795 (53.3)
Last Dental Visit, count (%)								
Within last 6 months	1,361 (75.9)	661 (65.6)	306 (45.7)	798 (65.2)	706 (73.3)	342 (39.8)	205 (42.8)	138 (9.2)

6 months to 2 years ago	372 (20.8)	262 (26.0)	186 (27.8)	172 (22.5)	200 (20.8)	232 (27.0)	112 (23.4)	216 (14.4)
2 to 5 years ago	48 (2.7)	56 (5.6)	100 (15.0)	58 (7.6)	40 (4.2)	143 (16.7)	92 (19.2)	325 (21.7)
More than 5 years ago	12 (0.7)	28 (2.8)	77 (11.5)	36 (4.7)	17 (1.8)	142 (16.5)	70 (14.6)	817 (54.6)
Dentist Visit, count (%)								
Regular Basis	1,656 (92.4)	850 (84.3)	323 (48.3)	552 (72.1)	842 (87.4)	352 (41.0)	215 (44.5)	91 (6.1)
Discomfort/Repair	130 (7.3)	141 (14.0)	319 (47.7)	196 (25.6)	114 (11.8)	473 (55.1)	237 (49.1)	1,172 (78.0)
Don't go to Dentist	1 (0.1)	6 (0.6)	19 (2.8)	8 (1.0)	1 (0.1)	20 (2.3)	24 (5.0)	227 (15.1)
Other	6 (0.3)	11 (1.1)	8 (1.2)	10 (1.3)	6 (0.6)	14 (1.6)	7 (1.5)	12 (0.8)

PPC-A: Health
PPC-B: Mild Disease
PPC-C: High Gingival Inflammation Index
PPC-D: Tooth Loss
PPC-E: Posterior Disease
PPC-F: Severe tooth Loss
PPC-G: Severe Disease

Significant associations were observed between self-reported periodontal disease and both clinical periodontal disease definitions (p<.0001). The cross tabulations between exposure categories and the corresponding chi squared p-values are shown in Tables 9, 10, and 11 below.

Table 9. Cross tabulation between CDC/AAP periodontal disease classification and self-reported history of tooth loss due to gum disease of ARIC participants at baseline (Visit 4 – 1996-1998)

	Self Report		
CDC/AAP	No	Yes	P-Value
Healthy	705 (95.0)	37 (5.0)	<.0001
Mild	1,863 (96.0)	78 (4.0)	
Moderate	2,406 (90.9)	241 (9.1)	
Severe	848 (77.9)	241 (22.1)	
Edentulous	962 (65.8)	601 (34.2)	

Table 10. Cross tabulation between periodontal profile classification and self-reported history of tooth loss due to gum disease of ARIC participants at baseline (Visit 4 – 1996-1998)

	Self-Report		
PPC	No	Yes	P-Value
A	1,719 (96.7)	58 (3.3)	<.0001
B	972 (97.5)	25 (2.5)	
C	574 (88.9)	72 (11.2)	
D	629 (84.4)	116 (15.6)	
E	855 (90.1)	94 (9.9)	
F	669 (79.4)	174 (20.6)	
G	404 (87.5)	58 (12.6)	
Edentulous	962 (65.8)	501 (34.2)	

PPC-A: Health
PPC-B: Mild Disease
PPC-C: High Gingival Inflammation Index
PPC-D: Tooth Loss
PPC-E: Posterior Disease
PPC-F: Severe tooth Loss
PPC-G: Severe Disease

Table 11. Cross tabulation between CDC/AAP periodontal disease classification and periodontal profile classification of ARIC participants at baseline (Visit 4 – 1996-1998)

PPC	Healthy	Mild	Moderate	Severe	Edentulous	P-Value
A	340 (18.9)	850 (47.1)	569 (31.6)	44 (2.4)	0 (0.0)	<.0001
B	90 (8.9)	389 (38.4)	467 (46.2)	66 (6.5)	0 (0.0)	
C	28 (4.2)	277 (41.1)	280 (41.5)	89 (13.2)	0 (0.0)	
D	74 (9.6)	194 (25.2)	356 (46.3)	145 (18.9)	0 (0.0)	
E	0 (0.0)	49 (5.1)	557 (57.7)	359 (37.2)	0 (0.0)	
F	218 (25.2)	199 (23.0)	314 (36.3)	135 (15.6)	0 (0.0)	
G	0 (0.0)	18 (3.7)	165 (34.0)	302 (62.3)	0 (0.0)	
Edentulous	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1,545 (100.0)	

PPC-A: Health
PPC-B: Mild Disease
PPC-C: High Gingival Inflammation Index
PPC-D: Tooth Loss
PPC-E: Posterior Disease
PPC-F: Severe tooth Loss
PPC-G: Severe Disease

Table 12 below contains the mean baseline (visit 4) CRP levels by periodontal disease status. Mean CRP levels at baseline were lower among those who did not report a history of tooth loss due to gum disease compared to those who did ($p=0.02$). Among CDC/AAP clinical periodontal levels, only edentulous had a significantly higher baseline CRP level ($p<0.0001$). Comparing baseline CRP by PPC classifications, only classifications D (tooth loss) and edentulous had consistently higher mean CRP levels compared to lower levels of periodontal disease ($p<0.05$).

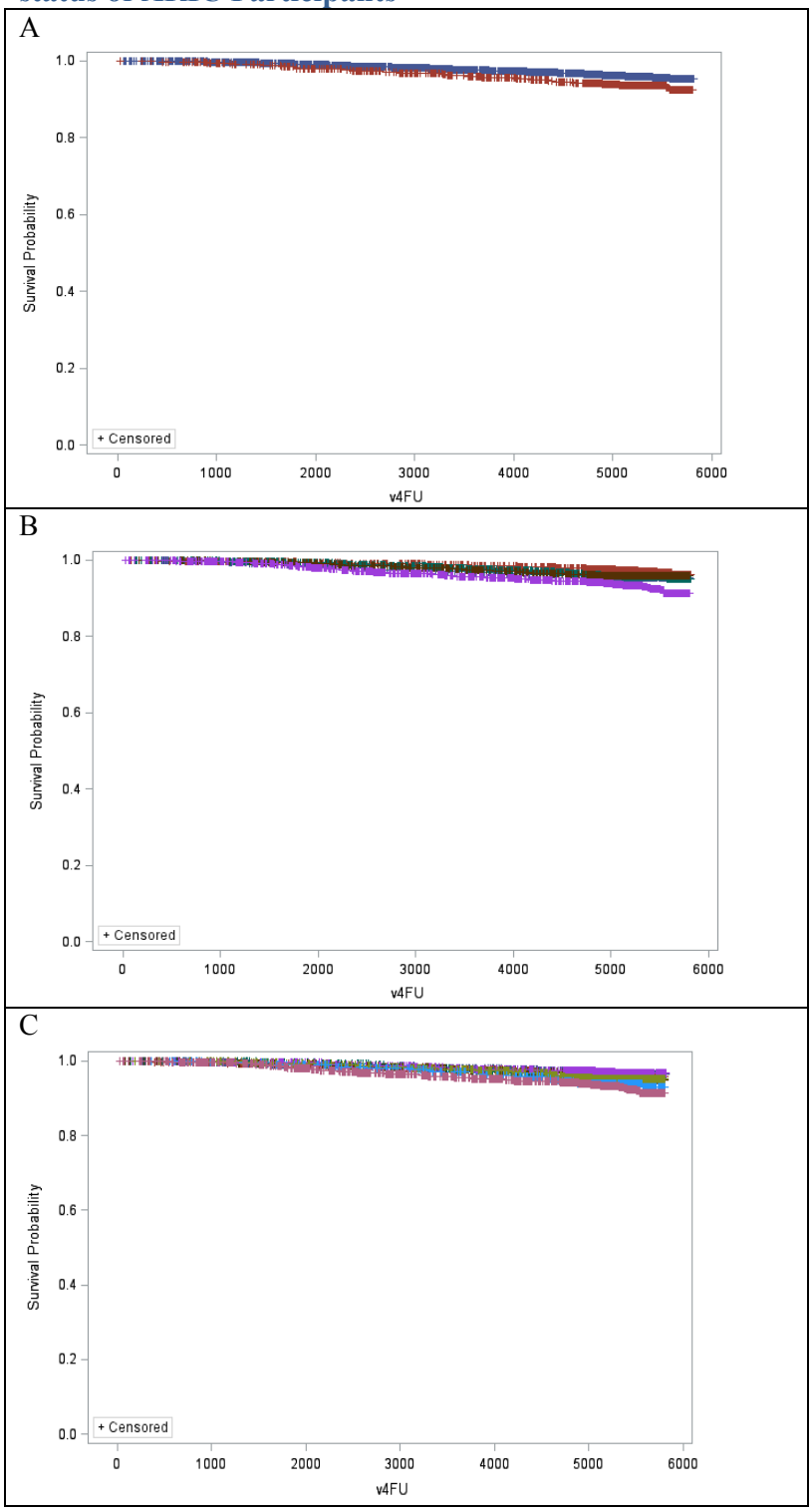
Table 12. C-reactive protein (CRP) level (mean and standard error) by periodontal disease status of ARIC Participants at Baseline (Visit 4 – 1996-1998)

Periodontal Infection	Adjusted Mean (mg/L)	Standard Error
Self-Report		
No	4.57	0.12
Yes	5.06*	0.21
CDC/AAP		
0	4.30	0.26
1	4.26	0.17
2	4.22	0.16
3	4.61	0.21
Edentulous	5.91*	0.19
PPC		
A	3.91	0.19
B	3.97	0.23
C	4.18	0.28
D	4.94*	0.24
E	4.03	0.24
F	4.76	0.24
G	4.74	0.91
Edentulous	5.91*	0.19

Adjusted for age, sex, and race/center
* Statistically different than lower levels ($p<0.05$)
PPC-A: Health
PPC-B: Mild Disease
PPC-C: High Gingival Inflammation Index
PPC-D: Tooth Loss
PPC-E: Posterior Disease
PPC-F: Severe tooth Loss
PPC-G: Severe Disease

The proportional hazards assumption was assessed by visual inspection of the Kaplan-Meier (KM) curves contained in Figure 6 below and by testing the interaction between periodontal disease status and follow-up time. The KM curves revealed no significant departures from proportionality. Further, no significant interactions between exposure and follow-up time were observed (all p-values >.05).

Figure 6. Kaplan-Meier survival curves for incident VTE by periodontal disease status of ARIC Participants



A. KM curve by self-reported periodontal disease
B. KM curve by CDC/AAP clinical periodontal disease status
C. KM curve by PPC clinical periodontal disease classification

Table 13 contains the results of the Cox proportional hazards regression models. Self-reported tooth loss due to periodontal disease was associated with higher risk of VTE. This association remained significant or borderline significant after adjustment for potential confounders. After adjustment, self-reported tooth loss due to gum disease was associated with 30% higher VTE risk compared to those who did not report past tooth loss due to gum disease (HR = 1.3 (1.0, 1.73)). Crude associations between CDC/AAP and PPC periodontal disease were observed but these associations were attenuated with adjustment and produced no statistically significant results.

Results from the sensitivity analysis in which we excluded participants who reported a history of endodontic therapy did not significantly differ from the primary results and are presented in Table 14 below.

Table 13. Association (HR and 95% CI) between self-reported and clinical periodontal disease and incident VTE in the ARIC cohort

	Events	N	Crude	Model 1	Model 2	Model 3
Self-Report						
No	246	6,784	Ref	Ref	Ref	Ref
Yes	61	1,098	1.64 (1.24, 2.17)	1.36 (1.02, 1.81)	1.30 (0.97, 1.74)	1.29 (0.96, 1.73)
CDC/AAP						
Healthy	31	750	Ref	Ref	Ref	Ref
Mild	51	1,976	0.61 (0.39, 0.95)	0.59 (0.37, 0.93)	0.59 (0.38, 0.94)	0.60 (0.38, 0.95)
Moderate	103	2,708	0.93 (0.52, 1.39)	0.89 (0.59, 1.33)	0.86 (0.57, 1.29)	0.86 (0.57, 1.29)
Severe	41	1,140	0.92 (0.57, 1.46)	0.77 (0.48, 1.25)	0.73 (0.45, 1.18)	0.71 (0.43, 1.15)
Edentulous	88	1,545	1.59 (1.06, 2.39)	1.09 (0.71, 1.68)	1.00 (0.65, 1.54)	1.02 (0.63, 1.63)
PPC						
A	53	1,803	Ref	Ref	Ref	Ref
B	34	1,012	1.16 (0.76, 1.79)	1.00 (0.64, 1.55)	0.94 (0.60, 1.46)	0.90 (0.58, 1.40)
C	28	674	1.45 (0.92, 2.29)	0.84 (0.49, 1.42)	0.74 (0.43, 1.25)	0.70 (0.41, 1.20)
D	25	769	1.15 (0.71, 1.85)	0.85 (0.52, 1.38)	0.74 (0.45, 1.21)	0.71 (0.43, 1.17)
E	26	965	0.95 (0.59, 1.52)	0.90 (0.56, 1.45)	0.82 (0.51, 1.33)	0.81 (0.50, 1.31)
F	35	866	1.46 (0.95, 2.24)	0.95 (0.60, 1.51)	0.81 (0.51, 1.29)	0.75 (0.46, 1.21)
G	25	485	1.90 (1.18, 3.06)	1.20 (0.71, 2.02)	1.05 (0.62, 1.77)	0.90 (0.52, 1.57)
Edentulous	88	1,545	2.29 (1.63, 3.22)	1.31 (0.88, 1.95)	1.09 (0.73, 1.64)	1.03 (0.64, 1.65)

Model 1: Included age, sex, race/center, education, income
Model 2: Added smoking, alcohol consumption, BMI, and statin use
Model 3: Added health insurance status, last dental visit, dental visit frequency, and having a current dentist
PPC-A: Health
PPC-B: Mild Disease
PPC-C: High Gingival Inflammation Index
PPC-D: Tooth Loss
PPC-E: Posterior Disease
PPC-F: Severe tooth Loss
PPC-G: Severe Disease

Table 14. Association (HR and 95% CI) between self-reported and clinical periodontal disease and incident VTE excluding those with self-reported history of endodontic therapy in the ARIC cohort

	Crude	Model 1	Model 2	Model 3
Self-Report				
No	Ref	Ref	Ref	Ref
Yes	1.61 (1.16, 2.23)	1.33 (0.95, 1.86)	1.27 (0.91, 1.79)	1.26 (0.90, 1.77)
CDC/AAP				
Healthy	Ref	Ref	Ref	Ref
Mild	0.51 (0.28, 0.94)	0.49 (0.27, 0.91)	0.51 (0.28, 0.94)	0.52 (0.28, 0.96)
Moderate	0.99 (0.59, 1.65)	0.93 (0.55, 1.56)	0.90 (0.53, 1.51)	0.91 (0.54, 1.53)
Severe	0.67 (0.35, 1.26)	0.55 (0.29, 1.05)	0.51 (0.27, 0.99)	0.49 (0.25, 0.95)
Edentulous	1.37 (0.83, 2.26)	0.97 (0.58, 1.63)	0.89 (0.52, 1.49)	0.86 (0.49, 1.50)
PPC				
A	Ref	Ref	Ref	Ref
B	1.01 (0.54, 1.89)	1.00 (0.64, 1.55)	0.83 (0.44, 1.58)	0.80 (0.42, 1.51)
C	1.43 (0.79, 2.61)	0.84 (0.49, 1.42)	0.66 (0.34, 1.31)	0.59 (0.30, 1.19)
D	1.30 (0.67, 2.55)	0.85 (0.52, 1.38)	0.75 (0.37, 1.50)	0.69 (0.34, 1.40)
E	0.82 (0.45, 1.88)	0.90 (0.56, 1.45)	0.75 (0.37, 1.55)	0.74 (0.36, 1.52)
F	1.42 (0.82, 2.47)	0.95 (0.60, 1.51)	0.71 (0.39, 1.30)	0.62 (0.33, 1.17)
G	1.42 (0.74, 2.74)	1.20 (0.71, 2.02)	0.75 (0.37, 1.52)	0.62 (0.29, 1.31)
Edentulous	2.06 (1.31, 3.23)	1.31 (0.88, 1.95)	0.94 (0.56, 1.57)	0.79 (0.43, 1.43)

Model 1: Included age, sex, race/center, education, income
Model 2: Added smoking, alcohol consumption, BMI, and statin use
Model 3: Added health insurance status, last dental visit, dental visit frequency, and having a current dentist
PPC-A: Health
PPC-B: Mild Disease
PPC-C: High Gingival Inflammation Index
PPC-D: Tooth Loss
PPC-E: Posterior Disease
PPC-F: Severe tooth Loss
PPC-G: Severe Disease

Discussion

We conducted a prospective cohort study on the relationship between periodontal disease and incident VTE using data from ARIC and the D-ARIC and LITE ancillary studies. We found that self-reported tooth loss due to gum disease was associated with 30% higher VTE risk and remained significant or borderline significant after adjustment for confounding. Crude associations between CDC/AAP and PPC clinical periodontal disease classifications were attenuated with adjustment and were no longer significant.

Our results differ from the previous study evaluating periodontal disease and VTE.[93] In that study, Sanchez-Siles et al. found that periodontitis was more prevalent among 97 VTE patients than in the 100 healthy controls ($p<.001$). The small sample size and case-control study design used in this study may make these results prone to bias. Our failure to replicate these findings suggests that further research on the association between periodontal disease and VTE is needed.

The discrepancy between our self-reported periodontal disease and clinical periodontal disease results is worth exploring. The mechanisms connecting infection and VTE may help explain the absence of an association between clinical periodontal disease and VTE. It is possible that the mechanisms connecting infection and VTE (inflammation and hypercoagulability) may operate acutely and may be less relevant with chronic infections like periodontal disease. Existing studies have shown that infection is associated with acute VTE risk but that the increase in risk decreases over time since infection.[121] The increase in inflammation and hypercoagulability associated with infection may not be sustained over the course of a chronic infection like periodontal

disease and may explain the lack of association between clinical periodontal disease and VTE.

Self-reported tooth loss due to gum disease may indicate more severe periodontal disease over a longer period of time compared to the clinical disease classifications which were categorized based on measures taken at a single point in time. The significant difference in baseline CRP levels between self-reported periodontal disease classifications that was absent between clinical periodontal disease classifications may support this explanation. The potential duration and severity associated with the self-reported exposure classification may explain the significant result that is absent in the clinical periodontal disease classifications.

While studies investigating the periodontal disease treatment as a primary prevention strategy of CVD are lacking, a number of studies have investigated the impact of periodontal treatment on CVD risk factors.[180] A recent meta-analysis of clinical trials investigating the impact of periodontal treatment on CVD risk factors found that periodontal treatment was effective at reducing systemic inflammatory marker levels and improving lipid profiles in subjects with periodontititis.[151] Further research is needed to determine if periodontal treatment reduces VTE risk.

Our study has a number of strengths, including a large sample size from a community cohort with lengthy follow-up and rigorous methodology to adjudicate VTE events. It also has limitations. Since we are only assessing exposure at a single time point, our study could suffer from measurement error since participant's periodontal disease status could change between baseline and the time of their event. Changes in periodontal disease status after baseline could not be accounted for and could create misclassification

of our exposure groups. This potential time lag between exposure and the outcome of interest would most likely lead to non-differential misclassification which could bias our results towards the null and may explain the absence of significant findings. Further, there could be measurement error associated with self-reported tooth loss due to gum disease and residual confounding due to the inability to include unmeasured confounders. The vast majority of VTE events were symptomatic since events were captured via hospital records and were clinically diagnosed and verified by the investigators without specific screening for asymptotic VTE events.

Conclusion

Self-reported tooth loss due to periodontal disease was associated with a 30% increase in VTE risk. The CDC/AAP and PPC clinical periodontal disease classifications were not associated with VTE risk. Further research is needed to elucidate the periodontal disease-VTE relationship.

Chapter 5 – Manuscript #2: Endodontic Therapy and Cardiovascular Disease: The Atherosclerosis Risk in Communities Cohort

Abstract

Background

Existing studies of an association between endodontic infection (EI) and cardiovascular disease (CVD) have produced mixed results. The majority of studies have focused the relationship between EI and coronary heart disease (CHD) while few studies have considered the relationship between EI and ischemic stroke (IS), heart failure (HF), or venous thromboembolism (VTE). EI may be related to CVD through infection-induced inflammation that contributes to atherosclerotic development and hypercoagulability. We hypothesized that EI is independently associated with CHD, IS, HF, and VTE risk.

Methods

We used a prospective cohort study design using longitudinal data from the Atherosclerosis Risk in Communities (ARIC) study, the Longitudinal Investigation of Thromboembolism Etiology (LITE) ancillary study, and the Dental-ARIC (D-ARIC) ancillary study to study the relationship between EI and incident CHD, IS, HF, and VTE. In the absence of radiographic information, EI was assessed through participants' self-reports of past endodontic therapy (ET). Cox-proportional hazards regression models were used to estimate hazard ratios and 95% confidence intervals for CHD, IS, HF, and VTE across ET classifications. Adjusted models were constructed to include relevant confounders including demographic and SES factors, CVD risk factors, and dental hygiene and access to care variables.

Results

No significant associations between self-reported history of ET and any of our outcomes of interest (CHD, IS, HF, VTE) were observed after adjustment for confounding.

Conclusion

Further research is needed to determine if EI is independently associated with CVD risk and if EI prevention and treatment could reduce CVD risk.

Introduction

Endodontic infection (EI) is infection of the dental root canal system in the pulp of the tooth and the major etiologic agent of apical periodontitis (AP) or infection of the apex of the tooth root.[181] EI/AP is relatively common but previous estimates of prevalence in western countries have varied between 14% and 70%.[28] A recent study conducted in Finland found that 27% of adults had one of more teeth with EI/AP.[99] Most epidemiologic studies that have explored the association between dental infections and cardiovascular disease (CVD) have focused on periodontal disease. Overall, they have found positive associations between periodontal disease and CVD.[82]

The primary mechanism linking both EI/AP and periodontitis and CVD includes systemic inflammation that leads to both atherosclerotic development and platelet aggregation and hypercoagulability.[82] Existing studies have shown that both EI/AP and periodontitis are associated with systemic inflammatory markers levels including interleukin-6 (IL-6)[133, 182-184], C-reactive protein (CRP)[133, 134, 184], and soluble intercellular adhesion molecule-1 (sICAM-1)[135]. These inflammatory markers have also been associated with increased risk of CVD and venous thromboembolism (VTE).[134, 142, 165, 166] The relationship between endodontic infection and CVD has not been studied as extensively but EI/AP may be related to CVD and VTE through these same mechanisms.[94]

In 2009, Caplan et al used data from the Atherosclerosis Risk in Communities study (ARIC) and the dental ancillary study (D-ARIC) to show a cross-sectional association between self-reported history of root canal therapy and prevalent CHD.[94] Since then, several other studies evaluating EI/AP and CVD have been published with mixed

results.[185] A recent systematic review found 19 studies that evaluated the association between apical periodontitis and CVD.[186] While 13 of the 19 studies found a positive association between AP and CVD, 5 found no association and 1 study found a negative association.[186] The authors conclude that there is considerable heterogeneity among previous studies in terms of study design, study population, outcomes of interest, and AP evaluation methods that has resulted in a lack of quality evidence of a causal relationship.[186]

Clinically, EI/AP is diagnosed through observation of periapical bony lesions on radiographs (indicative of chronic endodontic inflammation) or of radiopaque material in the root canal system (indicative of a history of root canal therapy).[94] In the absence of radiographic information, one way to estimate history of EI/AP is through participants' self-reports of endodontic therapy (ET).

Few previous studies have directly addressed the association between ET and the longitudinal development of stroke, heart failure (HF), or VTE. Therefore, we used longitudinal data from ARIC and the dental ancillary study (D-ARIC) to test the hypothesis that ET is independently associated with risk of incident CHD, incident stroke, incident VTE, and incident heart failure. We further hypothesized that the association would be graded such that those with multiple root canals will be at highest risk of incident CVD/VTE events.

Methods

The ARIC study is a multi-center population-based prospective cohort study designed to investigate the etiology and natural history of atherosclerosis in middle-aged Americans.[158] At baseline in 1987-1989 (visit 1), 15,792 white and black men and

women were enrolled from 4 US communities: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland.[158] Subsequent clinic exams took place during 1990 to 1992 (visit 2), 1993 to 1995 (visit 3), 1996 to 1998 (visit 4), 2011 to 2013 (visit 5), and 2016-2017(visit 6 currently underway) with continuously surveillance for CVD events. A detailed discussion of the ARIC study design, methods, and objectives is provided elsewhere.[158]

The purpose of D-ARIC was to determine the prevalence, extent, and severity of periodontal conditions in the dentate ARIC population and describe the associations between those conditions and prevalent CHD and atherosclerosis.[48] The D-ARIC study was an ARIC ancillary study that took place among ARIC participants at all 4 study sites during visit 4 (1996-1998). All ARIC participants who had natural teeth and who had no contraindications to or required antibiotic prophylaxis for periodontal probing were eligible to participate in D-ARIC. Details of the D-ARIC study objectives and methodology have been described in a previous publication.[162]

We used a prospective cohort study design in which visit 4 (1996-1998) served as the baseline for these analyses. All ARIC participants who completed the dental history questionnaire and who reported being edentulous at exam 4 (n=1,599) or who completed the D-ARIC exam (n=6,644) were included in the analysis. Those of races other than black and white (27) and those taking anticoagulants at baseline (visit 4) (100) were excluded from the analysis. Finally, those with the CVD outcome of interest at baseline as determined by self-report at study entry or adjudicated CVD events occurring prior to baseline (visit 4) were excluded from the respective analyses. Our final sample sizes after

exclusions were n=7,705 for CHD, n=7,026 for ischemic stroke, n=7,630 for HF, and n=7,925 for VTE.

Endodontic Therapy Ascertainment

The exposure of interest was self-reported history of root canal therapy. Exposure was classified according to responses to the questions, "Have you ever had root canal therapy?" and "(If you have had root canal therapy), Have you had more than one?" Exposure was trichotomized as multiple root canals, one root canal, and no root canals according to question responses.

Covariate Ascertainment

Demographic variables including age at visit 4, sex, race, and study center were assessed at baseline (visit 4) or updated from information collected at study enrollment (visit 1). Education (some high school or less, high school diploma, college degree or higher) was assessed at study entry (visit 1) while income (<$25,000/year, $25,000 – <$50,000/year, $50,000 – <$75,000/year, >$75,000/year) was assessed at baseline (visit 4). Common risk factors including smoking (current, former, never), diabetes (yes/no), hypertension (yes/no), LDL (mg/dL), HDL (mg/dL), triglycerides (mg/dL), statin use (yes/no), BMI (Kg/M^2),and alcohol consumption (grams/week) were assessed at baseline (visit 4). Finally, oral hygiene and access to care variables including medical insurance status (private insurance, Medicare/Medicaid only, none), last dental visit (<6 months ago, 6 months – <2 years ago, 2 – <5 years ago, >5 years ago), dental visit frequency (regularly, only for discomfort or repair, don't regularly visit the dentist), and having a current dentist (yes/no) were collected during the dental history questionnaire administered at baseline (visit 4). Baseline (visit 4) C-reactive protein (CRP) levels were

measured in 2008 on plasma frozen at −70°C from visit 4 by the immunoturbidimetric assay using the Siemens (Dade Behring) BNII analyzer (Dade Behring, Deerfield, Ill), performed according to the manufacturer's protocol.

Outcome Ascertainment

The outcomes of interest were incident CHD, incident ischemic stroke, incident heart failure, and incident VTE. Each outcome was analyzed separately. The methods used for ascertainment of outcomes included: (1) participants were contacted annually by phone and interviewed about interim hospitalizations; (2) local hospitals provided lists of hospital discharges with cardiovascular diagnoses, and these were reviewed to identify cohort hospitalizations; and (3) health department death certificate files were regularly surveyed. All discharge codes for cohort hospitalizations and listed causes of death from death certificates were recorded. CVD events were classified by a combination of computer algorithm and adjudicated physician review; disagreements were adjudicated by the ARIC Mortality and Morbidity Classification Committee using standardized ARIC criteria.[159]

Incident CHD was defined as confirmed CHD death, and fatal and nonfatal myocardial infarction (MI).[187] Incident ischemic stroke was identified and classified as thrombotic or cardioembolic stroke based on discharge codes, signs, symptoms, neuroimaging (computerized tomography/magnetic resonance imaging), and other diagnostic reports.[188] Both CHD and stroke events were validated by study physician review.

Incident heart failure (HF) was defined as the first occurrence of either (1) a hospitalization which included an International Classification of Diseases, 9th revision, discharge code of 428 (428.0 to 428.9) in any position, or (2) a death certificate with a 428 (HF) or ICD-10 code I50 (HF) in any position and was not adjudicated by physician review.[189]

Incident VTE was defined as all PEs and DVTs occurring in the legs and was identified using diagnosis codes, hospital records, physician and consultant reports, and discharge summaries and was validated according to LITE study protocol.[168] VTE events were validated by LITE study physician review.

Statistical Analysis

Descriptive statistics were calculated for study participant characteristics at baseline (visit 4) by endodontic therapy (ET) status. Baseline (visit 4) C-reactive protein (CRP) levels by ET status were compared using linear regression controlling for age, race/center, and sex. Means were compared using the least squares means procedure in SAS 9.3. Each outcome of interest was analyzed separately using Cox-proportional hazards regression models to estimate hazard ratios and 95% confidence intervals between ET categories. Those who reported having had a single root canal therapy and those having had multiple root canal therapies were compared against those without prior root canal therapy (referent). The proportional hazards assumption was assessed by visual inspection of the Kaplan-Meier (KM) survival curves and by testing the interaction between root canal therapy status and follow-up time.

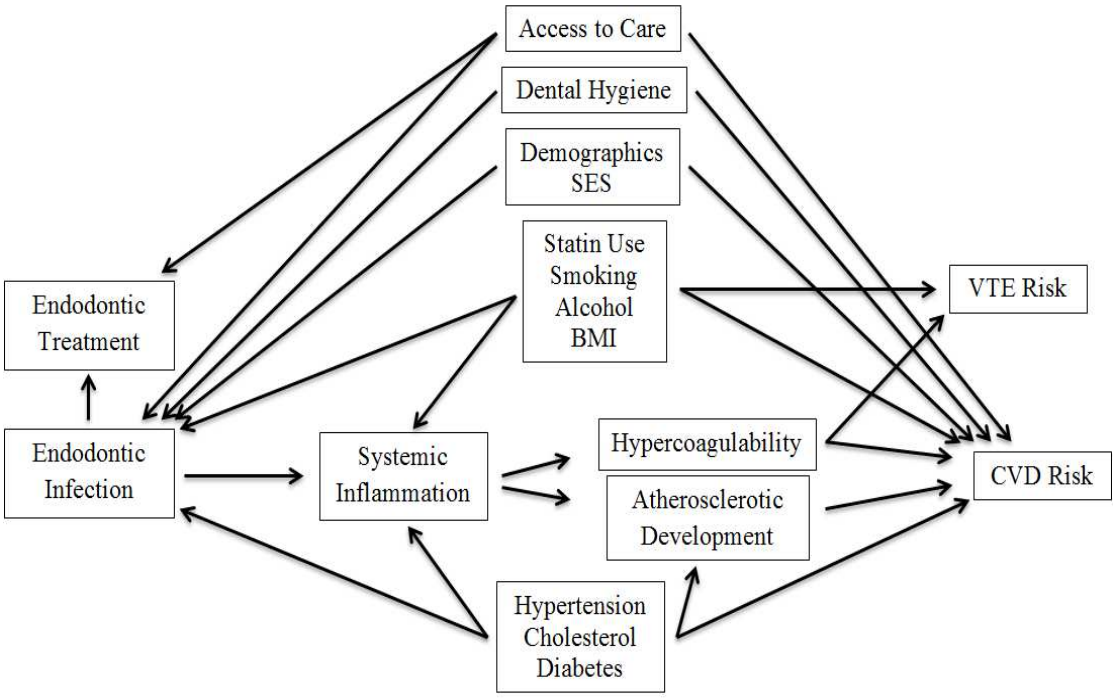
Crude (unadjusted) models and those adjusting for potential confounders were constructed. Covariate values were taken from assessments performed at the ARIC visit 4

exam. Only risk factors common to both oral infection and CVD were considered. Variable selection and model construction was informed by the causal diagram presented in Figure 7 below. In addition to crude models, model 1 included demographic and SES variables including age, sex, race/center, education, and income. Model 2 added adjustment for relevant oral infection risk factors that are also associated with CVD including smoking,[170, 171, 190, 191] alcohol consumption,[172, 173, 191] BMI,[174, 175, 191] statin use,[176, 177, 191, 192] diabetes,[191, 193] hypertension,[191, 193, 194] LDL,[151, 191, 192, 195] HDL,[151, 191, 192, 195] and triglycerides.[151, 191, 192, 195] Hypertension, diabetes, LDL, HDL, and triglycerides were excluded from the VTE analysis due to the lack of an independent association between these factors and VTE.[170] Finally, model 3 additionally included adjustment for variables related to oral hygiene and access to care including medical insurance status, last dentist visit, dental visit frequency, and having a current dentist.

Those who reported never having had ET consist of two highly disparate subgroups: those who had good oral health and never needed a root canal and those who had poor oral health and needed root canals but never received them. Since number of teeth is a good proxy for historical access to dental care, we conducted analyses stratified by the median number of teeth (24) similar to the approach used by Caplan et al.[94] This allowed us to evaluate the potential impact of ET among those with 25 or more teeth who likely had access to care and received root canal therapy when needed.

Finally, we performed a sensitivity analysis in which the association between ET and CVD was considered excluding participants who reported a history of tooth loss due to gum disease since periodontal infection may trigger a similar inflammatory response as endodontic infection.

Figure 7. Causal diagram of pathways linking endodontic infection and cardiovascular disease and venous thromboembolism risk



Follow- up time began at entry into the study (visit 4) and extended to the first outcome, dropping out of the study, death, or else, December 31, 2013 for CHD, stroke, and HF and December 31, 2011 for VTE.

Power and Sample Size

These analyses were conducted using previously collected observational data for which the sample size is fixed. With a fixed sample size, the question of interest became what is the minimum detectable effect (MDE) of ET on CVD for our study. Since the study considered multiple outcomes including CHD, ischemic stroke, heart failure, and VTE, we focused the MDE analysis on the VTE analysis since VTE is the rarest outcome of interest. The exposure of interest was ET categorized as no ET, 1 ET, and >1 ET. Since our primary analysis was stratified by median number of teeth (24), we considered

the MDE of the stratified analysis. The frequency of each exposure classification by teeth strata is found in Table 15 below.

Table 15. Frequency of endodontic therapy classifications at baseline by number of teeth strata

Endodontic Therapy	<25 Teeth (N)	≥25 Teeth (N)
0	3,128	1,474
1	686	732
≥2	990	915

Since those with multiple ETs and those with a single ET were compared to those without previous ET, we focused our MDE analysis on participants with ≥25 teeth comparing those with a single ET and to those without a previous ET since those with a single ET made up the smallest exposure classification and thus the lowest powered comparison with the largest MDE. We also compared those with multiple ETs to those without previous ET since they represented the most extreme exposure categories.

The MDE analyses were performed using the Power and Sample Size Program.[178, 179] Our study had 732 participants with a single ET and 915 participants with multiple ETs compared to 1,474 participants without a previous ET approximately 6% of whom will experience a VTE event during follow-up. Based on these criteria, Table 16 below contains MDE HRs for α-level (type I error rate) equal to 0.05 across levels of power (1-β).

Table 16. Minimum detect effects (HRs) of endodontic therapy on VTE by power levels:

ET	Power = 80%	Power = 85%	Power = 90%
1	1.54	1.58	1.64
≥2	1.48	1.51	1.56

By convention, we used 80% power, giving us a MDE = HR = 1.54 and 1.48 for those with a single ET and those with multiple ETs compared to those without a previous

ET respectively. This effect size is consistent with previous studies[51, 81] and suggests that the anticipated and meaningful effect size is detectable by our study.

Results

Baseline (ARIC visit 4) characteristics are provided in Table 17 below for study participants by self-reported ET status. In general, ET was more common among whites compared to blacks and among those of high SES status compared to those of low SES status. Those with past ET were less likely to be smokers and have diabetes compared to those without a history of ET. Finally, those with past ET were more likely to have medical insurance, a current dentist, and visit the dentist regularly.

Table 17. Baseline (visit 4 – 1996-1998) characteristics of ARIC participants by endodontic therapy status

	Root Canal Therapy		
	None	1 Treatment	≥ 2 Treatments
Total, count (%)	4,728	1,442	1,946
Age (years), mean ± SD	62.9 (5.7)	62.7 (5.7)	62.4 (5.4)
Sex, count (%)			
Male	2,101 (44.4)	626 (43.4)	916 (47.1)
Female	2,627 (55.6)	816 (56.6)	1,030 (52.9)
Race, count (%)			
White	3,322 (70.3)	1,263 (87.6)	1,775 (91.2)
Black	1,406 (29.7)	179 (12.4)	171 (8.8)
Education, count (%)			
Some High School	1,312 (27.8)	132 (9.2)	136 (7.0)
High School Diploma	1,981 (42.0)	637 (44.2)	829 (42.7)
Bachelor's or Graduate Degree	1,428 (30.3)	673 (46.7)	975 (50.3)
Income, count (%)			
<$24,999	1,815 (39.6)	282 (19.8)	318 (16.5)
$25,000-$50,000	1,493 (32.5)	537 (37.7)	747 (38.7)
$50,000-$74,999	690 (15.0)	322 (22.6)	417 (21.6)
>$75,000	478 (10.4)	256 (17.9)	415 (21.5)
Refused	112 (2.4)	27 (1.9)	34 (1.8)
Smoker, count (%)			
Current	790 (16.8)	175 (12.2)	249 (12.8)
Former	1,919 (40.9)	627 (43.6)	950 (48.9)
Never	1,984 (42.3)	636 (44.2)	743 (38.3)
Diabetes mellitus, count (%)			
No	3,768 (80.4)	1,262 (88.1)	1,697 (87.7)
Yes	917 (19.6)	170 (11.9)	239 (12.4)
Hypertension, count (%)			
No	2,774 (59.0)	962 (66.9)	1,330 (68.5)
Yes	1,927 (41.0)	476 (33.1)	611 (31.5)
LDL (mg/dL), mean ± SD	3.20 (0.97)	3.15 (0.85)	3.14 (0.82)
HDL (mg/dL), mean ± SD	1.28 (0.42)	1.33 (0.45)	1.28 (0.47)
Triglycerides (mg/dL), mean ± SD	1.61 (0.98)	1.62 (0.93)	1.67 (1.01)
Statin Use, count (%)			
No	4,197 (89.2)	1,288 (89.4)	1,716 (88.2)
Yes	511 (10.9)	152 (10.6)	229 (11.8)
BMI (Kg/M^2), mean ± SD	29.1 (5.9)	28.1 (5.0)	28.4 (5.1)
Alcohol (g/week), mean ± SD	28.9 (76.0)	38.8 (83.1)	38.2 (82.4)
Care Payment, count (%)			
Health Plan	3,769 (80.1)	1,345 (93.5)	1,826 (93.9)
Medicare/Medicaid Only	564 (12.0)	56 (3.9)	64 (3.3)
None	369 (7.9)	37 (2.6)	54 (2.9)
Current Dentist, count (%)			
Yes	3,380 (71.8)	1,352 (94.0)	1,829 (94.2)
No	1,330 (28.2)	87 (6.1)	112 (5.8)
Last Dental Visit, count (%)			
Within last 6 months	1,131 (24.0)	50 (3.5)	53 (2.7)

6 months to 2 years ago	679 (14.4)	87 (6.0)	101 (5.2)
2 to 5 years ago	1,003 (21.3)	339 (23.5)	423 (21.8)
More than 5 years ago	1,897 (40.3)	964 (66.9)	1,368 (70.3)
Dental Visit, count (%)			
Regular Basis	2,159 (45.7)	1,156 (80.2)	1,574 (80.9)
Discomfort/Something Fixed	2,217 (46.9)	268 (18.6)	345 (17.7)
Don't go to Dentist	293 (6.2)	9 (0.6)	14 (0.7)
Other	54 (1.1)	8 (0.6)	12 (0.6)

Table 18 below contains the mean baseline (visit 4) CRP levels by ET status overall and stratified by the median number of teeth. No significant differences were present in mean CRP levels at baseline between ET categories ($p > 0.05$).

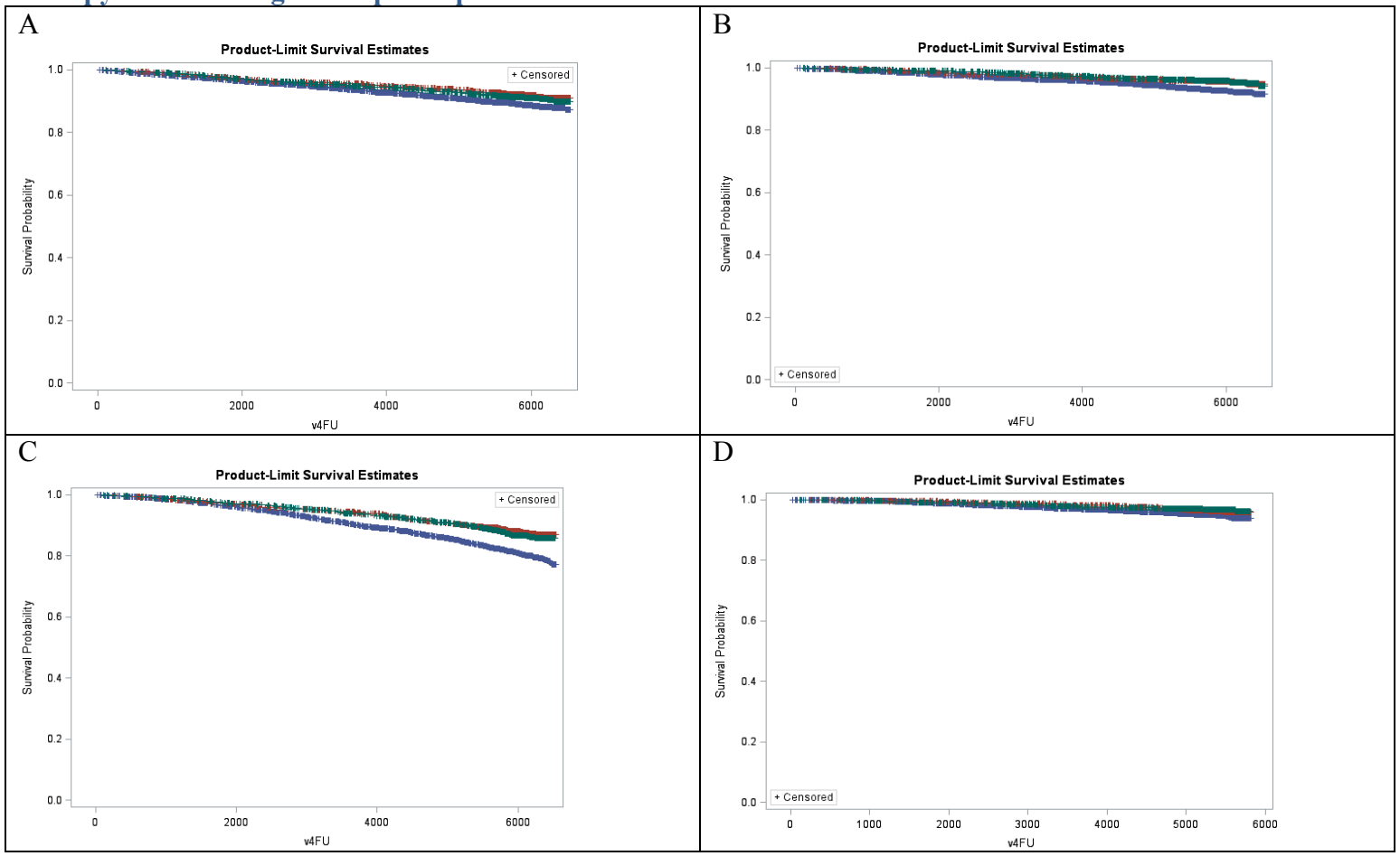
Table 18. C-reactive protein (CRP) level (mean and standard error) by endodontic therapy status overall, and stratified by number of teeth of ARIC participants at baseline (visit 4 – 1996-1998)

Endodontic Therapy	Adjusted* Mean (mg/L)	Standard Error
All Participants		
None	4.74	0.13
1 Treatment	4.51	0.20
≥ 2 Treatments	4.48	018
≥ 25 Teeth		
None	3.78	0.23
1 Treatment	3.95	0.28
≥ 2 Treatments	3.81	0.27
≤ 24 Teeth		
None	5.09	0.17
1 Treatment	4.79	0.29
≥ 2 Treatments	4.77	0.26

* Adjusted for age, sex, and race/center

The proportional hazards assumption was assessed by visual inspection of the Kaplan-Meier (KM) curves contained in Figure 8 below and by testing the interaction between periodontal disease status and follow-up time. The KM curves revealed no significant departures from proportionality. Further, no significant interactions between endodontic therapy status and follow-up time were observed (all p-values > 0.05).

Figure 8. Kaplan-Meier survival curves for incident CHD, ischemic stroke, heart failure, and VTE by endodontic therapy status among ARIC participants



A - CHD
B - Stroke
C - HF
D – VTE

Table 19 contains the results of the Cox proportional hazards regression models for ET and CHD. Crude models for all participants showed that those with a single ET and those with multiple ETs had lower CHD risk that was attenuated with adjustment for confounders and was no longer statistically significant. Among those with more than 24 teeth, both single ET (HR = 1.1 (0.7, 1.6)) and multiple ETs (HR = 1.1 (0.8, 1.6)) were associated with higher CHD risk after adjustment for confounding but these associations failed to reach statistical significance.

Ischemic stroke results are found in Table 20 below. Crude models from the combined analysis showed lower stroke risk among both those with a single ET and those with multiple ETs that was attenuated with adjustment for confounders and was no longer significant. In the stratified analysis, among those with more than 24 teeth, no associations between single ET (HR = 0.9 (0.6, 1.5) and multiple ETs (HR = 0.9 (0.5, 1.4)) and ischemic stroke were observed.

Table 21 contains the results of the Cox proportional hazards regression models for ET and HF. The crude models from the unstratified analysis showed that ET was associated with lower HF risk that was attenuated with adjustment for confounders and was no longer statistically significant. Among those with more than 24 teeth, both crude and adjusted models showed no association between ET and HF for both single ET (HR = 0.8 (0.6, 1.1)) and multiple ETs (HR = 0.8 (0.6, 1.1).

Table 22 contains the Cox proportional hazards regression model results for ET and VTE. In the crude models, ET was associated with lower VTE risk that was attenuated with adjustment and was no longer significant. The stratified analysis showed that the multiple ETs category was associated with higher VTE risk in those with more

than 24 teeth (HR = 1.2 (0.6, 1.6) but this association failed to reach statistical significance.

Results from the sensitivity analysis in which we excluded participants who reported a history of tooth loss due to gum disease did not significantly differ from the primary results and are presented in Tables 23-26 below.

Table 19. Association (hazard ratio and 95% confidence interval) between self-reported endodontic therapy and incident coronary heart disease in the ARIC cohort, overall and stratified by the median number of teeth

Endodontic Therapy	Events	N	Crude Model	Model 1	Model 2	Model 3
None	446	4,452	Ref	Ref	Ref	Ref
1 Treatment	105	1,397	0.71 (0.57, 0.88)	0.85 (0.68, 1.06)	0.96 (0.76, 1.20)	1.01 (0.80, 1.28)
≥ 2 Treatments	154	1,856	0.79 (0.66, 0.95)	0.97 (0.80, 1.18)	1.07 (0.87, 1.31)	1.11 (0.90, 1.37)
≥ 25 Teeth						
None	91	1,457	Ref	Ref	Ref	Ref
1 Treatment	43	729	0.94 (0.66, 1.36)	0.99 (0.69, 1.43)	1.09 (0.74, 1.58)	1.06 (0.73, 1.55)
≥ 2 Treatments	57	891	1.01 (0.72, 1.40)	1.09 (0.78, 1.53)	1.10 (0.77, 1.56)	1.09 (0.77, 1.55)
≤ 24 Teeth						
None	355	2,995	Ref	Ref	Ref	Ref
1 Treatment	62	668	0.73 (0.56, 0.95)	0.79 (0.59, 1.05)	0.89 (0.66, 1.18)	0.97 (0.72, 1.31)
≥ 2 Treatments	97	965	0.81 (0.64, 1.01)	0.87 (0.68, 1.12)	1.02 (0.79, 1.31)	1.08 (0.83, 1.42)

Model 1 Included age, sex, race/center, education, and income
Model 2 Added smoking, diabetes, alcohol consumption, BMI, hypertension, LDL, HDL, triglycerides, statin use
Model 3 Added usual medical care payment mechanism, last dental visit, dental visit frequency, and having a current dentist

Table 20. Association (hazard ratio and 95% confidence interval) between self-reported endodontic therapy and incident ischemic stroke in the ARIC cohort, overall and stratified by the median number of teeth

Endodontic Therapy	Events	N	Crude Model	Model 1	Model 2	Model 3
None	292	4,598	Ref	Ref	Ref	Ref
1 Treatment	58	1,417	0.60 (0.46, 0.80)	0.76 (0.57, 1.02)	0.80 (0.59, 1.08)	0.83 (0.61, 1.14)
≥ 2 Treatments	75	1,911	0.58 (0.45, 0.75)	0.78 (0.59, 1.02)	0.82 (0.62, 1.08)	0.84 (0.63, 1.12)
≥ 25 Teeth						
0 ET	59	1,466	Ref	Ref	Ref	Ref
1 ET	23	734	0.77 (0.48, 1.25)	0.82 (0.51, 1.34)	0.90 (0.54, 1.49)	0.91 (0.55, 1.52)
≥ 2 ET	28	913	0.75 (0.48, 1.17)	0.83 (0.53, 1.32)	0.87 (0.54, 1.39)	0.85 (0.53, 1.37)
≤ 24 Teeth						
0 ET	233	3,132	Ref	Ref	Ref	Ref
1 ET	35	683	0.63 (0.45, 0.91)	0.75 (0.52, 1.09)	0.76 (0.53, 1.14)	0.80 (0.54, 1.19)
≥ 2 ET	47	998	0.60 (0.44, 0.81)	0.73 (0.52, 1.02)	0.77 (0.54, 1.09)	0.80 (0.56, 1.15)

Model 1 Included age, sex, race/center, education, and income
Model 2 Added smoking, diabetes, alcohol consumption, BMI, hypertension, LDL, HDL, triglycerides, statin use
Model 3 Added usual medical care payment mechanism, last dental visit, dental visit frequency, and having a current dentist

Table 21. Association (hazard ratio and 95% confidence interval) between self-reported endodontic therapy and incident heart failure in the ARIC cohort, overall and stratified by the median number of teeth

Endodontic Therapy	Events	N	Crude Model	Model 1	Model 2	Model 3
None	740	4,406	Ref	Ref	Ref	Ref
1 Treatment	150	1,376	0.60 (0.51, 0.72)	0.77 (0.64, 0.93)	0.85 (0.70, 1.02)	0.93 (0.77, 1.13)
≥ 2 Treatments	217	1,848	0.65 (0.56, 0.76)	0.89 (0.77, 1.05)	0.92 (0.77, 1.09)	1.01 (0.85, 1.21)
≥ 25 Teeth						
None	145	1,444	Ref	Ref	Ref	Ref
1 Treatment	54	718	0.75 (0.55, 1.02)	0.78 (0.57, 1.07)	0.79 (0.57, 1.09)	0.79 (0.58, 1.10)
≥ 2 Treatments	67	885	0.73 (0.55, 0.98)	0.81 (0.60, 1.09)	0.78 (0.57, 1.06)	0.79 (0.58, 1.07)
≤ 24 Teeth						
None	595	2,962	Ref	Ref	Ref	Ref
1 Treatment	96	658	0.66 (0.53, 0.82)	0.80 (0.64, 1.01)	0.87 (0.69, 1.10)	0.99 (0.77, 1.27)
≥ 2 Treatments	150	963	0.72 (0.60, 0.86)	0.94 (0.77, 1.14)	1.00 (0.81, 1.22)	1.14 (0.91, 1.41)

Model 1 Included age, sex, race/center, education, and income
Model 2 Added smoking, diabetes, alcohol consumption, BMI, hypertension, LDL, HDL, triglycerides, statin use
Model 3 Added usual medical care payment mechanism, last dental visit, dental visit frequency, and having a current dentist

Table 22. Association (hazard ratio and 95% confidence interval) between self-reported endodontic therapy and incident venous thromboembolism in the ARIC cohort, overall and stratified by the median number of teeth

Endodontic Therapy	Events	N	Crude Model	Model 1	Model 2	Model 3
None	203	4,602	Ref	Ref	Ref	Ref
1 Treatment	46	1,418	0.69 (0.50, 0.95)	0.83 (0.59, 1.17)	0.86 (0.61, 1.21)	0.90 (0.64, 1.28)
≥ 2 Treatments	56	1,905	0.63 (0.47, 0.85)	0.85 (0.62, 1.17)	0.87 (0.63, 1.20)	0.92 (0.66, 1.27)
≥ 25 Teeth						
None	47	1,474	Ref	Ref	Ref	Ref
1 Treatment	19	732	0.81 (0.47, 1.37)	0.83 (0.49, 1.43)	0.85 (0.49, 1.45)	0.86 (0.50, 1.48)
≥ 2 Treatments	31	915	1.05 (0.67, 1.65)	1.12 (0.71, 1.79)	1.17 (0.73, 1.87)	1.20 (0.75, 1.92)
≤ 24 Teeth						
None	156	3,128	Ref	Ref	Ref	Ref
1 Treatment	27	686	0.73 (0.48, 1.09)	0.86 (0.56, 1.34)	0.91 (0.58, 1.41)	1.00 (0.63, 1.56)
≥ 2 Treatments	25	990	0.48 (0.31, 0.72)	0.68 (0.43, 1.07)	0.70 (0.45, 1.11)	0.79 (0.50, 1.26)

Model 1 Included age, sex, race/center, education, income
Model 2 Added smoking, alcohol consumption, BMI, statin use
Model 3 Added usual medical care payment mechanism, last dental visit, dental visit frequency, and having a current dentist

Table 23. Association (hazard ratio and 95% confidence Interval) between self-reported endodontic therapy and incident coronary heart disease in the ARIC cohort excluding participants who self-reported tooth loss due to gum disease, overall and stratified by median number of teeth

Endodontic Therapy	Crude Model	Model 1	Model 2	Model 3
None	Ref	Ref	Ref	Ref
1 Treatment	0.73 (0.58, 0.92)	0.86 (0.68, 1.09)	0.98 (0.76, 1.25)	1.03 (0.80, 1.33)
≥ 2 Treatments	0.81 (0.66, 0.99)	0.99 (0.80, 1.23)	1.08 (0.87, 1.35)	1.13 (0.90, 1.43)
≥ 25 Teeth				
None	Ref	Ref	Ref	Ref
1 Treatment	0.91 (0.62, 1.33)	0.94 (0.64, 1.37)	1.01 (0.68, 1.50)	1.00 (0.67, 1.48)
≥ 2 Treatments	0.97 (0.69, 1.37)	1.04 (0.73, 1.48)	1.04 (0.72, 1.50)	1.04 (0.72, 1.50)
≤ 24 Teeth				
None	Ref	Ref	Ref	Ref
1 Treatment	0.75 (0.56, 1.02)	0.83 (0.61, 1.14)	0.96 (0.70, 1.32)	1.05 (0.75, 1.47)
≥ 2 Treatments	0.84 (0.65, 1.08)	0.93 (0.70, 1.22)	1.08 (0.82, 1.44)	1.17 (0.87, 1.58)

Model 1 Included age, sex, race/center, education, and income
Model 2 Added smoking, diabetes, alcohol consumption, BMI, hypertension, LDL, HDL, triglycerides, statin use
Model 3 Added usual medical care payment mechanism, last dental visit, dental visit frequency, and having a current dentist

Table 24. Association (hazard ratio and 95% confidence Interval) between self-reported endodontic therapy and incident ischemic stroke in the ARIC cohort excluding participants who self-reported tooth loss due to gum disease, overall and stratified by median number of teeth

Endodontic Therapy	Crude Model	Model 1	Model 2	Model 3
None	Ref	Ref	Ref	Ref
1 Treatment	0.61 (0.45, 0.83)	0.78 (0.57, 1.07)	0.82 (0.59, 1.14)	0.88 (0.63, 1.23)
≥ 2 Treatments	0.63 (0.48, 0.82)	0.84 (0.63, 1.11)	0.89 (0.66, 1.19)	0.93 (0.69, 1.27)
≥ 25 Teeth				
None	Ref	Ref	Ref	Ref
1 Treatment	0.79 (0.48, 1.30)	0.83 (0.51, 1.38)	0.91 (0.54, 1.53)	0.93 (0.55, 1.57)
≥ 2 Treatments	0.80 (0.51, 1.26)	0.89 (0.56, 1.42)	0.94 (0.59, 1.52)	0.92 (0.57, 1.50)
≤ 24 Teeth				
None	Ref	Ref	Ref	Ref
1 Treatment	0.63 (0.43, 0.94)	0.76 (0.51, 1.15)	0.79 (0.51, 1.20)	0.86 (0.55, 1.33)
≥ 2 Treatments	0.63 (0.45, 0.88)	0.78 (0.54, 1.12)	0.82 (0.56, 1.20)	0.90 (0.61, 1.33)

Model 1 Included age, sex, race/center, education, and income
Model 2 Added smoking, diabetes, alcohol consumption, BMI, hypertension, LDL, HDL, triglycerides, statin use
Model 3 Added usual medical care payment mechanism, last dental visit, dental visit frequency, and having a current dentist

Table 25. Association (hazard ratio and 95% confidence Interval) between self-reported endodontic therapy and incident heart failure in the ARIC cohort excluding participants who self-reported tooth loss due to gum disease, overall and stratified by median number of teeth

Endodontic Therapy	Crude Model	Model 1	Model 2	Model 3
None	Ref	Ref	Ref	Ref
1 Treatment	0.59 (0.49, 0.72)	0.75 (0.62, 0.92)	0.81 (0.66, 1.00)	0.89 (0.72, 1.10)
≥ 2 Treatments	0.65 (0.55, 0.77)	0.88 (0.73, 1.05)	0.89 (0.74, 1.07)	0.97 (0.80, 1.18)
≥ 25 Teeth				
None	Ref	Ref	Ref	Ref
1 Treatment	0.76 (0.55, 1.05)	0.77 (0.56, 1.07)	0.78 (0.56, 1.09)	0.79 (0.56, 1.09)
≥ 2 Treatments	0.74 (0.55, 1.00)	0.80 (0.59, 1.09)	0.78 (0.57, 1.08)	0.79 (0.58, 1.09)
≤ 24 Teeth				
None	Ref	Ref	Ref	Ref
1 Treatment	0.62 (0.48, 0.79)	0.78 (0.60, 1.01)	0.83 (0.63, 1.09)	0.93 (0.70, 1.24)
≥ 2 Treatments	0.70 (0.57, 0.86)	0.93 (0.74, 1.16)	0.97 (0.76, 1.22)	1.11 (0.86, 1.42)

Model 1 Included age, sex, race/center, education, and income
Model 2 Added smoking, diabetes, alcohol consumption, BMI, hypertension, LDL, HDL, triglycerides, statin use
Model 3 Added usual medical care payment mechanism, last dental visit, dental visit frequency, and having a current dentist

Table 26. Association (hazard ratio and 95% confidence Interval) between self-reported endodontic therapy and incident venous thromboembolism in the ARIC cohort excluding participants who self-reported tooth loss due to gum disease, overall and stratified by median number of teeth

Endodontic Therapy	Crude Model	Model 1	Model 2	Model 3
None	Ref	Ref	Ref	Ref
1 Treatment	0.72 (0.51, 1.02)	0.87 (0.60, 1.26)	0.89 (0.62, 1.29)	0.92 (0.63, 1.34)
≥ 2 Treatments	0.66 (0.48, 0.91)	0.88 (0.62, 1.24)	0.89 (0.63, 1.26)	0.92 (0.64, 1.31)
≥ 25 Teeth				
None	Ref	Ref	Ref	Ref
1 Treatment	0.83 (0.48, 1.41)	0.84 (0.49, 1.44)	0.85 (0.49, 1.46)	0.87 (0.51, 1.50)
≥ 2 Treatments	0.96 (0.60, 1.54)	1.02 (0.63, 1.64)	1.05 (0.65, 1.70)	1.08 (0.67, 1.76)
≤ 24 Teeth				
None	Ref	Ref	Ref	Ref
1 Treatment	0.72 (0.45, 1.16)	0.93 (0.56, 1.55)	0.96 (0.58, 1.59)	1.05 (0.62, 1.77)
≥ 2 Treatments	0.50 (0.31, 0.81)	0.77 (0.46, 1.29)	0.79 (0.47, 1.32)	0.87 (0.51, 1.48)

Model 1 Included age, sex, race/center, education, income
Model 2 Added smoking, alcohol consumption, BMI, statin use
Model 3 Added usual medical care payment mechanism, last dental visit, dental visit frequency, and having a current dentist

Discussion

We conducted a prospective cohort study on the relationship between self-reported history of endodontic therapy and incident CHD, incident stroke, incident HF, and incident VTE using data from ARIC and the D-ARIC and LITE ancillary studies. We found no association between self-reported history of ET and any of our outcomes of interest that remained after adjustment for confounding in either the overall analysis or among those with more than 24 teeth in the stratified analysis. Our results differ from the previous studies that identified a positive association between EI/AP and CHD[94, 101-103, 107-110] but are consistent with other previous studies that found no association between EI/AP and CHD.[47, 111]

The discrepancy between our results and the majority of previous studies is worth exploring. We hypothesized an independent association between EI/AP and CVD. This hypothesis was based upon previous research linking EI/AP to CHD and previous studies that identified an association between periodontal infection and CVD through mechanisms that may also operate between EI/AP and CVD. The absence of an association ET and our CVD outcomes of interest may suggest that despite the similarities between EI/AP and periodontal disease, they may impact the cardiovascular system differently.

In 2009, Caplan et al conducted a cross-sectional study using ARIC data and found that among participants with 25 or more teeth, those reporting having had ET two or more times had 1.62 times higher odds of CHD compared with those reporting never having had ET.[94] This and other previous studies that used cross-sectional and case-control study designs to identify associations between EI/AP and CHD cannot assess the

temporality between EI/AP and CHD and may be more prone to bias such as reverse causation not present in our longitudinal study.

Previous cohort studies on EI/AP and CHD have found mixed results. Gomes et al found an association between endodontic burden and cardiovascular events ((RR = 1.77, 95% CI = 1.04-3.02) but found no association between apical periodontitis, root canal therapy, or oral inflammatory burden and incident cardiovascular events.[102] An earlier study by Caplan et al found that among those ≤ 40 years old, endodontic lesions were significantly associated with incident CHD ($p < 0.05$) but found no association among those > 40 years old.[107]A study from 2001 by Jansson et al found that apical lesions were significantly associated with death due to CVD ($p<0.05$) however, only age and gender were included in the adjusted models.[196]

Our study has a number of other strengths including a large sample size from a community cohort with lengthy follow-up and rigorous methodology to adjudicate CVD events. Our study also has a number of potential limitations that could result in failing to detect a potential association between EI/AP and CVD. Our study could be susceptible to measurement error since EI/AP exposure was assessed using self-reported ET at a single point in time (baseline) as a proxy for EI/AP. ET could be performed for restorative reasons such as prophylaxis in trauma cases and not endodontic reasons. Also, a lack of ET does not necessarily imply the absence of EI/AP since teeth could be extracted or remain asymptomatic. The lack of significant differences in baseline CRP levels between ET classifications may support this explanation. Since exposure was only assessed at a single point in time, ET that occurred during follow up was not accounted for and could create misclassification of our exposure groups. A potential time lag between exposure

and our outcomes of interest may result in non-differential misclassification which could bias our results towards to null. Misclassification of exposure could take place since historical ET may be forgotten or mistaken for other procedures.

Two studies have evaluated the validity of self-reported history of ET compared to radiographic verified ET. Pitiphat et al. compared self-reported measures of ET obtained by a self-administered questionnaire with measures simultaneously obtained with clinical and radiograph examinations. They found that self-reported history of root canal therapy had 90% sensitivity, 94% specificity, a positive predictive value of 86% and a negative predictive value of 95% among 58 adult patients to the Harvard School of Dental Medicine student dental clinic.[105] A more recent study conducted by Gomes et al compared self-reported history of ET with ET status determined simultaneously from panoramic radiographs. They found that self-reported history of ET had 92% sensitivity, 89% specificity, 82% positive predictive value, and 95% negative predictive value.[106] Both studies concluded that self-reported history of ET is a highly accurate method to predict historic ET.[105, 106]

Individuals who have not received or do not have access to dental care may not have received ET when it would be otherwise warranted. We attempted to isolate those with access to dental care by stratifying by the median number of teeth and including predictors of dental care in our adjusted models but measurement error could persist. Residual confounding from unmeasured confounders also cannot be ruled out.

Conclusion

ET was not associated with CHD, ischemic stroke, HF, or VTE in our study. Further research is needed to elucidate the EI/AP-CVD relationship.

Chapter 6 – Manuscript #3: Infection as a Trigger of Cardiovascular Disease: The Atherosclerosis Risk in Communities Cohort

Abstract

Background

There is evidence that infection triggers CVD events but the magnitude and duration of increased CVD risk following infection is not well understood. Additionally, little is known regarding outpatient infection as a CVD trigger. We hypothesized that both all infections and outpatient infections are independently associated with risk of CHD, ischemic stroke, and VTE and that the infection-CVD association will be stronger among total infections compared to outpatient infections and graded such that the association will be strongest immediately following the infection and decrease as the time since the infection increases.

Methods

CHD, ischemic stroke, and VTE cases were identified in the Atherosclerosis Risk in Communities (ARIC) cohort. Hospital discharge diagnosis codes were used to identify inpatient infections. Additionally, Medicare claims data linked to ARIC participants were used to identify both inpatient and outpatient infections. A case-crossover design and conditional logistic regression were used to compare infections among CHD, ischemic stroke, and VTE cases (14, 30, 42, and 90 days before the event) with corresponding control periods 1 year and 2 years prior.

Results

Infection was associated with higher odds of CHD (14-day OR, 3.8, 30-day OR, 2.9, 42-day OR, 2.6, and 90-day OR, 2.1), ischemic stroke (14-day OR, 2.6, 30-day OR, 1.9, 42-day OR, 1.8, and 90-day OR, 1.7), and VTE (14-day OR, 18.9, 30-day OR, 8.0, 42-

day OR, 5.7, and 90-day OR, 3.6) up to 90 days following infection. The association was strongest immediately following the infection and decreased as the time since the infection increased. Generally, outpatient infection was a weaker CVD trigger compared to all infections.

Conclusion

Patients with an infection should be considered potential candidates for CVD prophylaxis. Treatment with standard CVD preventive strategies, including antiplatelet agents and statins, should be considered during and immediately after infection to reduce the otherwise elevated CVD risk.

Introduction

Population-based cohort studies have identified many chronic risk factors for cardiovascular disease (CVD) that are both modifiable, such as high blood pressure, elevated serum cholesterol, and smoking, and non-modifiable, such as male sex, non-white race, family history, and greater age.[5, 6] Acute risk factors – or triggers – of CVD are less studied. Identifying and understanding CVD triggers offer potential strategies for CVD prevention during vulnerable periods.

Prior research has provided evidence that infection triggers acute CVD events including MI[114, 115, 197], stroke[114, 118, 119], and VTE.[124] While the results of these studies are informative, most previous studies only included hospitalized infections as their exposure of interest. Since outpatient encounters for infection are more than 5 times as common as inpatient encounters for infection in the United States, assessing the impact of outpatient infections on CVD will add to the body of knowledge.[11] Further, the magnitude and duration of increased cardiovascular risk has varied greatly between studies and remains under debate.

We expanded upon our prior work linking inpatient infection with stroke[118] and VTE[121] by using the longitudinal data from the Atherosclerosis Risk in Communities (ARIC) study and the corresponding ARIC participant data from the Centers for Medicare and Medicaid Services (CMS) to examine the relationship between infection (both inpatient and outpatient) and outpatient infection only and CVD. We hypothesized that both total infections and outpatient infections will be independently associated with risk of CHD, ischemic stroke, and VTE. We further hypothesized that the infection-CVD association will be stronger among total infections compared to outpatient infections

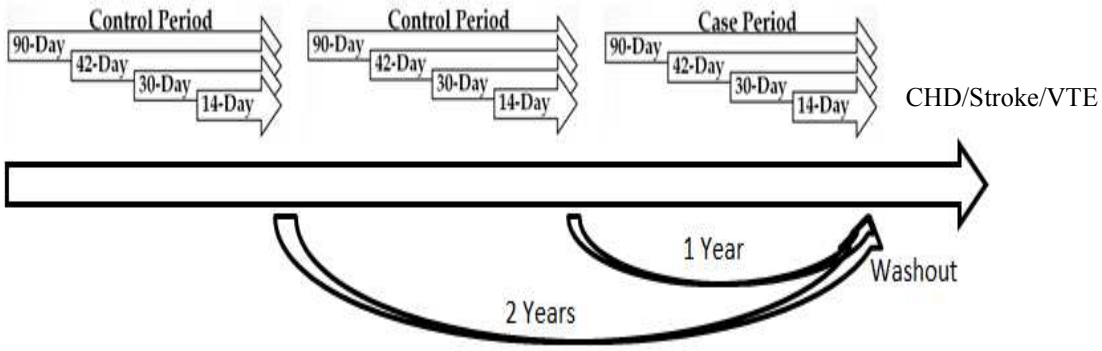
since inpatient infections are likely more severe and graded such that the infection-CVD association will be strongest immediately following the infection and decrease as the time since the infection increases.

Methods

Study Design

The ARIC study is a multi-center population-based prospective cohort study designed to investigate the etiology and natural history of atherosclerosis in middle-aged Americans.[158] At baseline in 1987-1989 (visit 1), 15,792 white and black men and women were selected from 4 US communities: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland.[158] Subsequent exams took place during 1990 to 1992 (visit 2), 1993 to 1995 (visit 3), 1996 to 1998 (visit 4), 2011 to 2013 (visit 5), and 2016-2017 (visit 6 currently underway). We used a case-crossover study design in which ARIC participants with CVD outcomes served as their own controls. The occurrence of infection immediately prior to CVD events was compared with preceding time intervals 1 year and 2 years prior to the CVD event. The crossover study design is summarized in Figure 9 below.

Figure 9. Case-crossover study design used to study infection as a CVD trigger, ARIC.



All ARIC participants with each outcome of interest during follow-up were included. CVD events were identified using information collected at each exam, annual telephone questionnaires, and hospitalizations. Hospitalizations were identified by surveillance of local hospital discharge lists for cohort members. Information obtained at study visits, during telephone questionnaires, and through review and abstraction of hospital and death records were used to adjudicate CVD outcomes and identify inpatient infections.[159] Additionally, CMS claims data were used to identify both inpatient and outpatient infections in ARIC study participants. CMS claims data for inpatient and outpatient services were available since 1991. We excluded individuals who were younger than 67 years of age at the time of their CVD event since they were not Medicare eligible for both the case and control periods. We also excluded participants whose CVD events occurred prior to 1993 to ensure that CMS data were available for both case and control periods.

Inpatient and Outpatient Infection Ascertainment

The exposure of interest was infection determined using ICD-9 codes. Inpatient hospitalization codes and outpatient visit codes were used to identify infections. Table 27 below contains the infection types and corresponding ICD-9 codes that were included in our exposure of interest.

Table 27. Infection type and corresponding ICD-9 codes included in the exposure of interest

Infection	ICD-9 Codes
Other Infectious Diseases	001-139
Thymus Gland Infection	254.1
Nervous System Infections	320-326, 331.81
Eye Infections	372–372.39, 373.0-373.2
Ear Infections	382-382.4, 383, 386.33, 386.35, 388.60
Circulatory Infections	390-393, 421-421.1, 422.0, 422.91-422.93
Respiratory Infections	460–466,472–474.0,475–476.1,478.21–478.24,478.29, 480-490, 491.1, 494, 510-511, 513.0, 518.6, 519.01
Digestive Infections	522.5, 522.7, 527.3, 528.3, 540–542, 566–567.9, 569.5, 572–572.1, 573.1–573.3, 575–575.12
Urinary Tract Infections	590–590.9, 595–595.4, 597–597.89, 598.0, 599.0
Male Genital Infections	601–601.9, 604–604.9, 607.1, 607.2, 608.0, 608.4
Breast Infections	611.0
Female Pelvic Infections	614–616.1, 616.3–616.4, 616.8
Puerperal Infections	670
Skin and Subcutaneous Infections	680–686.9, 706.0
Musculoskeletal Infections	711–711.9, 730–730.3, 730.8–730.9,
Blood Infections	790.7–790.8
Healthcare Acquired Infections	996.60–996.69, 997.62, 998.5, 999.3

Cardiovascular Events

The outcomes of interest were CHD, ischemic stroke, and VTE. The methods used for outcome ascertainment included: (1) participants were contacted annually by phone and interviewed about interim hospitalizations; (2) local hospitals provided lists of hospital discharges with cardiovascular diagnoses that were reviewed to identify cohort hospitalizations; and (3) health department death certificate files were regularly surveyed. All discharge codes for cohort hospitalizations and listed causes of death from death certificates were recorded. CVD events were classified by a combination of computer algorithm and adjudicated physician review; disagreements were adjudicated by the ARIC Mortality and Morbidity Classification Committee using standardized ARIC criteria.[159]

CHD was defined as confirmed CHD death and fatal and nonfatal MI.[187] Ischemic stroke was identified and classified as thrombotic or cardioembolic stroke based on

discharge codes, signs, symptoms, neuroimaging (computerized tomography/magnetic resonance imaging), and other diagnostic reports.[188] Hemorrhagic strokes were not included since no clear biological pathways mediating hemorrhagic stroke risk and infection have been proposed or identified.

VTE was defined as all PEs and DVTs and restricting DVTs to those that occurred in the leg and were identified using diagnosis codes, hospital records, physician and consultant reports, and discharge summaries validated according to LITE study protocol.[168]

Statistical Analysis

Each outcome of interest was analyzed separately. The prevalence of infection 14, 30, 42, and 90 days before CVD events was compared with the corresponding time periods exactly 1 year and 2 years before the event. Conditional logistic regression was used to estimate odds ratios (OR) of CVD events and 95% confidence intervals (CIs) for each time period (14, 30, 42, and 90 days). Separate models were run using all infections (both inpatient and outpatient) and outpatient infections only to see if the magnitude of the association differed between infection types.

Potential confounders that are stable within an individual are controlled in the case-crossover study design by having cases serve as their own controls. Confounding by overall health status related to age is possible because deteriorating health could be a common cause of both infection and CVD. As participants age and their health status decreases, their CVD risk and risk of infection may increase, suggesting potential positive confounding by health status.

To reduce potential confounding, only time periods proximal to the CVD event (1 year and 2 years before) were included. We further controlled for the total number of hospitalizations in the 9 months preceding the start of each of the 3 exposure periods (case period and 2 control periods) to account for potential decline in overall health status. Since hospitalization is a known VTE trigger, we further controlled for the total number of hospitalizations in the 90-day exposure period in the VTE analysis to account for non-infection triggering due to hospitalization and isolate the impact of infection.

Results

Among the 15,792 ARIC study participants, 2,356 (14.9%) experienced CHD (CHD death, fatal and nonfatal MI). Those who were younger than 67 years of age at the time of their event (1,017) and those events that occurred prior to 1993 (27) were excluded to ensure that CMS data were available for both case and control periods. Our final sample size was n=1,312 CHD cases.

A combined 1,150 (7.3%) ARIC participants experienced an ischemic stroke. We excluded those who were younger than 67 years of age at the time of their event (410) and those events that occurred prior to 1993 (13) to ensure that CMS data were available for both case and control periods. Our final sample size was n=727 ischemic stroke cases.

Only 845 (5.4%) ARIC participants experienced a VTE event and 755 (4.8%) experienced a PE or DVT in the leg. To ensure that CMS data were available for both case and control periods, we excluded those who were younger than 67 years of age at the time of their event (all - 270 and PE and leg DVT - 239) and those events that occurred prior to 1993 (all – 7 and PE and leg DVT - 6). Our final sample size was all VTE - n=568 cases, PE and leg DVT – n=510 cases.

At-event characteristics of ARIC participants who developed CHD, ischemic stroke, and VTE are provided in Table 28 below. The mean age at CVD event was 75 years. CHD was more common among men (57.4%) while ischemic stroke (54.1%) and VTE (56.0%) were more common among women. The majority of events occurred in white participants consistent with baseline enrollment.

Table 28. At-event characteristics of ARIC participants who developed CVD or VTE, 1987-2013

Characteristic	CHD (n=1,312)	Ischemic Stroke (n=727)	All VTE (n=568)	PE and Leg VTE (n=510)
Age, years, mean ± SD	75.0 (5.3)	75.1 (5.1)	74.7 (5.2)	74.7 (5.2)
Sex, count (%)				
Male	753 (57.4)	334 (45.9)	250 (44.0)	219 (42.9)
Female	559 (42.6)	393 (54.1)	318 (56.0)	291 (57.1)
Race, count (%)				
White	949 (72.3)	492 (67.7)	341 (60.0)	315 (61.8)
Black	359 (27.4)	234 (32.2)	227 (40.0)	195 (38.2)
Other	4 (0.3)	1 (0.1)	0 (0.0)	0 (0.0)

Of the 1,312 CHD cases, 402 (30.6%) had any infection and 366 (27.9%) had an outpatient infection in the 90 days preceding their CHD event. Table 29 below contains the conditional logistic regression model results for CHD. All infection and outpatient infection were more common in all case periods compared with equivalent control periods, but the ORs decreased with elapsed time: 14-day OR, 3.8 (95% CI, 2.9–5.0); 30-day OR, 2.9 (95% CI, 2.3–3.6); 42-day OR, 2.6 (95% CI, 2.1–3.2), and 90-day OR, 2.1 (95% CI, 1.8–2.6). Controlling for the number of hospitalizations in the 9 month period preceding each exposure period slightly attenuated the association between infection and CHD. When the exposure of interest was restricted to outpatient infections only, the association between infection and CHD was slightly attenuated but remained significant at all time points: 14-day OR, 3.3 (95% CI, 2.5, 4.3); 30-day OR, 2.7 (95% CI, 2.1–3.4); 42-day OR, 2.5 (95% CI, 2.0–3.1), and 90-day OR, 2.0 (95% CI, 1.6–2.4). For both all

infections and outpatient infections, the association was strongest in exposure periods closest to the CHD event and decreased as the time window before CHD increased.

Table 29. Association between infection and CHD in the ARIC Cohort, OR, (95% CI)

All Infections	Case (n)	Control (n)	Crude Model	Model 1
14 Days				
No Infection	1,135	2,514	Ref	Ref
Infection	177	110	3.91 (3.00, 5.10)	3.84 (2.94, 5.03)
30 Days				
No Infection	1,059	2,394	Ref	Ref
Infection	253	230	2.96 (2.38, 3.68)	2.90 (2.33, 3.61)
42 Days				
No Infection	1,022	2,317	Ref	Ref
Infection	290	307	2.67 (2.17, 3.28)	2.63 (2.14, 3.24)
90 Days				
No Infection	910	2,083	Ref	Ref
Infection	402	541	2.19 (1.81, 2.64)	2.14 (1.77, 2.58)
Outpatient Infections				
14 Days				
No Infection	1,159	2,517	Ref	Ref
Infection	153	107	3.35 (2.55, 4.50)	3.29 (2.50, 4.32)
30 Days				
No Infection	1,084	2,404	Ref	Ref
Infection	228	220	2.72 (2.17, 3.41)	2.69 (2.14, 3.37)
42 Days				
No Infection	1,052	2,333	Ref	Ref
Infection	260	291	2.47 (1.99, 3.06)	2.45 (1.97, 3.05)
90 Days				
No Infection	946	2,109	Ref	Ref
Infection	366	515	2.02 (1.67, 2.46)	1.99 (1.64, 2.42)

Model 1: Adjusted for total hospitalizations in the 9 months preceding each exposure period

Of the 727 ischemic stroke cases, 186 (25.6%) had any infection and 173 (23.8%) had an outpatient infection in the 90 days preceding the stroke event. Table 30 below contains the conditional logistic regression model results for infection and ischemic stroke. Infection was more common in all case periods compared with equivalent control

periods, but the ORs decreased with elapsed time: 14-day OR, 2.6 (1.8, 3.8); 30-day OR, 1.9 (1.4, 2.5); 42-day OR, 1.8 (1.3, 2.4), and 90-day OR, 1.7 (1.5, 2.2). Controlling for the number of hospitalizations in the 9 month period preceding each exposure period slightly attenuated the association between infection and ischemic stroke. The association between outpatient infection and ischemic stroke was slightly attenuated compared to all infections but remained significant at all time points: 14-day OR, 2.4 (1.7, 3.5); 30-day OR, 1.7 (1.2, 2.3); 42-day OR, 1.7 (1.3, 2.3), and 90-day OR, 1.7 (1.3, 2.2). Overall, the association was strongest in exposure periods closest to the stroke event and decreased as the time window before ischemic stroke increased.

Table 30. Association between infection and ischemic stroke in the ARIC Cohort, OR (95% CI)

All Infections	Case (n)	Control (n)	Crude Model	Model 1
14 Days				
No Infection	652	1,391	Ref	Ref
Infection	75	63	2.71 (1.88, 3.91)	2.63 (1.82, 3.80)
30 Days				
No Infection	624	1,329	Ref	Ref
Infection	103	125	1.93 (1.42, 2.62)	1.85 (1.36, 2.52)
42 Days				
No Infection	604	1,295	Ref	Ref
Infection	123	159	1.85 (1.39, 2.46)	1.78 (1.34, 2.38)
90 Days				
No Infection	541	1,188	Ref	Ref
Infection	186	266	1.79 (1.39, 2.30)	1.74 (1.5, 2.24)
Outpatient Infections				
14 Days				
No Infection	659	1,393	Ref	Ref
Infection	68	61	2.48 (1.71, 3.60)	2.42 (1.66, 3.53)
30 Days				
No Infection	634	1,334	Ref	Ref
Infection	93	120	1.77 (1.29, 2.41)	1.69 (1.23, 2.31)
42 Days				
No Infection	614	1,302	Ref	Ref
Infection	113	152	1.75 (1.31, 2.35)	1.68 (1.25, 2.26)
90 Days				
No Infection	554	1,203	Ref	Ref
Infection	173	251	1.74 (1.34, 2.26)	1.69 (1.30, 2.20)

Model 1: Adjusted for total hospitalizations in the 9 months preceding each exposure period

We observed 568 VTE cases with 270 (47.5%) total infections and 253 (44.5%) outpatient infections in the in the 90 days preceding the VTE event and 510 PE and leg DVT cases with 237 (46.5%) total infections and 223 (43.7%) outpatient infections in the 90 days preceding the PE or leg DVT event. Table 31 below contains the conditional logistic regression model results for infection and VTE. Infection was more common in

all case periods compared with equivalent control periods, but the ORs decrease with elapsed time: 14-day OR, 18.9 (10.4, 34.3); 30-day OR, 8.0 (5.4, 11.9); 42-day OR, 5.7 (4.0, 8.2), and 90-day OR, 3.6 (2.6, 5.0). Controlling for the number of hospitalizations in the 9 month period preceding each exposure period did not affect the association between infection and VTE. Controlling for the number of hospitalizations in the 90 day exposure period slightly attenuated the association. The association between outpatient infection and VTE was similar to the association between all infections at all time points: 14-day OR, 22.9 (12.2, 43.1); 30-day OR, 8.5 (5.7, 12.9); 42-day OR, 6.3 (4.3, 9.1), and 90-day OR, 3.8 (2.7, 5.2). Overall, the association was strongest in exposure periods closest to the VTE event and decreased as the time window before VTE increased. Similar results were found when the analysis was restricted to PEs and leg DVTs.

Table 31. Association between infection and venous thromboembolism in the ARIC Cohort OR, 95% CI

All Infections	Case (n)	Control (n)	Crude Model	Model 1	Model 2
All VTEs					
14 Days					
No Infection	388	1,092	Ref	Ref	Ref
Infection	180	44	21.35 (12.59, 35.23)	21.32 (12.46, 36.46)	18.85 (10.36, 34.3)
30 Days					
No Infection	347	1,044	Ref	Ref	Ref
Infection	221	92	11.09 (7.67, 16.05)	11.11 (7.64, 16.17)	7.97 (5.35, 11.88)
42 Days					
No Infection	338	1,012	Ref	Ref	Ref
Infection	230	124	8.54 (6.16, 11.93)	8.43 (6.01, 11.83)	5.70 (3.98, 8.16)
90 Days					
No Infection	298	910	Ref	Ref	Ref
Infection	270	226	5.82 (4.35, 7.80)	5.63 (4.19, 7.56)	3.62 (2.63, 4.97)
PEs & Leg DVTs					
14 Days					
No Infection	351	982	Ref	Ref	Ref
Infection	159	38	21.79 (12.35, 38.42)	21.66 (12.20, 38.46)	19.01 (9.96, 36.30)
30 Days					
No Infection	317	940	Ref	Ref	Ref
Infection	193	80	10.68 (7.24, 15.75)	10.66 (7.19, 15.79)	7.57 (4.97, 11.52)
42 Days					
No Infection	309	910	Ref	Ref	Ref
Infection	201	110	8.03 (5.67, 11.39)	7.94 (5.58, 11.30)	5.32 (3.65, 7.74)
90 Days					
No Infection	273	819	Ref	Ref	Ref
Infection	237	201	5.42 (4.00, 7.33)	5.27 (3.88, 7.14)	3.34 (2.40, 4.65)
Outpatient Infections	Case (n)	Control (n)	Crude Model	Model 1	Model 2
All VTEs					
14 Days					

No Infection	394	1,097	Ref	Ref	Ref
Infection	174	39	21.04 (12.40, 35.72)	21.25 (12.39, 36.45)	22.90 (12.16, 43.14)
30 Days					
No Infection	356	1,048	Ref	Ref	Ref
Infection	212	88	10.72 (7.40, 15.53)	10.73 (7.37, 15.63)	8.53 (5.67, 12.85)
42 Days					
No Infection	349	1,018	Ref	Ref	Ref
Infection	219	118	8.74 (6.19, 12.36)	8.68 (6.11, 12.33)	6.28 (4.32, 9.14)
90 Days					
No Infection	315	921	Ref	Ref	Ref
Infection	253	215	5.69 (4.21, 7.68)	5.54 (4.09, 7.50)	3.77 (2.72, 5.22)
PEs & Leg DVTs					
14 Days					
No Infection	356	985	Ref	Ref	Ref
Infection	154	35	19.94 (11.52, 34.52)	19.98 (11.44, 34.89)	21.16 (10.94, 40.90)
30 Days					
No Infection	325	941	Ref	Ref	Ref
Infection	185	79	9.65 (6.61, 14.11)	9.63 (6.56, 14.14)	7.37 (4.84, 11.21)
42 Days					
No Infection	318	914	Ref	Ref	Ref
Infection	192	106	7.88 (5.53, 11.23)	7.80 (5.45, 11.15)	5.56 (3.79, 8.17)
90 Days					
No Infection	287	827	Ref	Ref	Ref
Infection	223	193	5.15 (3.79, 7.00)	5.01 (3.68, 6.82)	3.37 (2.41, 4.71)

Model 1: Adjusted for total hospitalizations in the 9 months preceding each exposure period
Model 2: Adjusted for total hospitalizations in the 9 months and 90 days preceding each exposure period

Discussion

This case-crossover study within a population-based cohort demonstrated that CHD, ischemic stroke, and VTE risk is higher after infection. Patients with infection had higher odds of CHD, stroke, and VTE up to 90 days after the infection compared with equivalent control periods 1 year and 2 years before the event. This provides evidence in support of our hypothesis that infection is associated with higher acute CVD/VTE risk and that infection is a CVD/VTE trigger. Generally, the association between infection and CVD was weaker when only considering outpatient infections as we hypothesized since inpatient infections are likely more severe and trigger a stronger inflammatory response. The association between infection and CVD/VTE was also graded such that the infection-CVD/VTE association was highest in the exposure periods most proximal to the event and decreased as the time window before the event increased.

Our findings corroborate previous work that has identified a triggering association between infection and CVD and that the risk varies by time since infection. Considering infection as a trigger of CHD, our results are similar in magnitude to those found by Warren-Gash et al (IRR = 4.19)[115], Corrales-Medina et al (HR = 4.07)[18], and Dalager-Pedersen et al using hospital controls (RR = 2.32) but smaller than their analysis using population controls (30-day RR = 17.70)[114] and the cross-over results of Chew et al (OR = 7.5).[117]

Our reported associations between infection and ischemic stroke are smaller in magnitude compared to the study published by our group that considered the association between inpatient infections and ischemic stroke using ARIC data.[118] Our results are also

smaller effect sizes than those shown by Elkind et al (OR = 7.3) [119] and Fullerton et al (OR = 6.3).[120] Our results for infection as a trigger of VTE were generally stronger than previous studies including a study by our group (OR = 2.65)[121], Chen et al (OR = 1.9)[122], Dalager-Pedersen et al (DVT HR = 1.78, PE HR = 1.97)[123], and Rogers et al. (OR=2.90).[124] The unusually strong ORs in our VTE analysis are a function of the high percentage of VTE cases that had an infection prior to their event. Since VTE is often provoked by cancer, trauma, or immobility, these provoking factors could lead to diagnostic bias by which those with a provoking factor are more likely to detect and be diagnosed with an infection due to heightened surveillance and more frequent contact with healthcare providers. More research is needed to understand the magnitude of VTE risk following infection.

The primary mechanism linking infection and CVD is through systemic inflammation which acutely leads to platelet aggregation and hypercoagulability and chronically leads to atherosclerotic development.[82] Epaulard et al. summarized existing studies that showed that the inflammatory response to infection and the coagulation and fibrinolysis processes likely share common pathways, explaining why infection is associated with thrombosis and CVD.[198] Further, infection can disrupt endothelial function that can contribute to the development of and growth of atherosclerosis over time.[119] Infection may also lead to immobility which can contribute to thrombosis through stasis.

Our study has a number of strengths, including a large sample size from a community cohort, ascertainment of both inpatient and outpatient infection exposure

data, a rigorous methodology to adjudicate CVD events, and a crossover design to control for potential confounding. It also has limitations. Like all case crossover studies, our study may suffer from survival bias as we did not consider infections in participants who did not have a CVD event. Our study only considered the relationship between infection and CVD among those who survived any infections and later had a CVD event. Confounding by age is possible because as participants age their risk of both CVD and infection increase. However, to reduce potential confounding by age, only control periods 1 and 2 years prior to CVD events were examined. We also adjusted for possible confounding by the total number of hospitalizations in the 9 month period preceding each exposure period. Other confounders that may vary between the exposure and control periods were not included. Because we used the hospital admission date as the CVD event date, dates for patients who do not seek immediate medical attention may be inaccurate, but we think that this is rare because most patients immediately seek care. We may be under-ascertaining infections, especially minor ones that did not require care since exposure data were collected using hospital and medical claims data. This would most likely lead to non-differential misclassification of the exposure that would typically bias ORs toward the null.

Conclusion

CVD patients had higher odds of infection within 90 days prior to their CVD event compared to equivalent control periods 1 and 2 years previous. Both all infections and outpatient infections are triggers of CHD, ischemic stroke, and VTE but the triggering association may be weaker for outpatient infections.

Previous research on CVD triggers has referred to the time period immediately following an infection as a "treatable moment" that may hold an opportunity for CVD prevention.[119] Since inpatient infections appear to be stronger CVD triggers compared to outpatient infections, patients with an inpatient infection may be of particular interest for CVD prophylaxis. Previous work has also identified infection as a particularly strong CVD trigger among those with otherwise low CVD risk.[118, 119, 199] CVD prophylaxis following an infection may also be particularly relevant for these patients. There may be a role for infection in CVD prevention decision making, though clinical trials and a cost-benefit analysis should be considered.

Chapter 7 – Conclusion

There is a growing evidence of an association between infection and cardiovascular disease. The overall purpose of this dissertation was to improve the body of knowledge related to the relationship between infection and CVD. Specifically, to better understand the association between periodontal disease and incident VTE and endodontic infection and incident CHD, stroke, heart failure, and VTE; and to better understand the triggering effect of infection on CHD, stroke, and VTE.

In the first manuscript, we evaluated the impact of periodontal disease on VTE. We hypothesized that periodontal disease is independently associated with risk of incident VTE and that the risk is graded such that the association between periodontal disease and VTE is highest among those with more severe periodontal disease. We found an association between self-reported tooth loss due to gum disease and VTE that remained significant or borderline significant after adjustment for confounding while the crude associations between the clinical periodontal disease classifications were attenuated with adjustment and were no longer significant.

In the second manuscript, we explored a potential association between endodontic therapy and CHD, ischemic stroke, heart failure, and VTE. Our hypothesis was that ET is independently associated with risk of incident CHD, incident stroke, incident VTE, and incident heart failure and that the association between ET and CVD is graded such that those with multiple root canals will be at highest risk of incident CVD events. We found no evidence of an association between history of ET and any of our CVD outcomes of interest (CHD, ischemic stroke, HF, VTE) that remained after adjustment for

confounding in either the overall analysis or among those with more than 24 teeth in the stratified analysis.

The third manuscript evaluated the role of infection as a trigger of CHD, ischemic stroke, and VTE. We hypothesized that both total infections and outpatient infections are independently associated with risk of CHD, stroke, and VTE and that the infection-CVD association is stronger among total infections compared to outpatient infections only since inpatient infection are likely more severe and graded such that the association between infection and CVD will be strongest in the exposure periods most proximal to the CVD event and decrease as the time window before the event increases. Results from the third manuscript provide evidence that infection is a CVD trigger. We demonstrated that CHD, ischemic stroke, and VTE risk is higher up to 90 days after an infection and that the association between infection and CVD is graded such that CVD risk was highest in the exposure periods most proximal to the CVD event and decreased as the time window before the event increases. Generally, the association between infection and CVD was weaker when only considering outpatient infections as we hypothesized since inpatient infections are likely more severe.

The results from the three manuscripts collective have a number of implications. While prevention and treatment of periodontal infection has a variety of health benefits both from a public health and clinical perspective, our results are mixed as to whether these measures may reduce VTE risk. While studies investigating the treatment of periodontitis as a primary prevention strategy of CVD are lacking, a number of studies have investigated the impact of periodontal treatment on CVD risk factors.[180] A recent

meta-analysis of clinical trials investigating the impact of periodontal treatment of CVD risk factors found that periodontal treatment was effective at reducing levels of systemic inflammation markers and improving lipid profiles in subjects with periodontititis.[151] It is unclear from these findings and our mixed results if periodontal treatment could reduce VTE risk and further research is needed.

The prevention, diagnosis, and treatment of endodontic infection have a variety of important public health and clinical benefits. The results of our second manuscript do not support an association between ET and CVD. The relationship between endodontic infection and CVD is still under debate and it is unclear if prevention or treatment of endodontic infection as a CVD prevention strategy has merit. A recent systematic review found 19 studies that evaluated the association between apical periodontitis and CVD.[186] While 13 of the 19 studies found a positive association between apical periodontitis and CVD, 5 found no association and 1 study found a negative association.[186] The authors conclude that there is considerable heterogeneity among the previous studies in terms of their study design, study population, outcomes of interest and AP evaluation methods that has produced a lack of quality evidence of a causal relationship.[186] While our research has expanded the CVD outcomes considered, further research us needed to elucidate the endodontic infection-CVD relationship.

The results of the third manuscript provide evidence in support of our hypothesis that infection is a CVD trigger. Patients with an infection should be considered potential candidates for CVD prophylaxis. Identification of infection as a CVD trigger may prompt more aggressive treatment with standard CVD preventive strategies, including

antiplatelet agents and statins, during and immediately after infection to reduce the otherwise elevated CVD risk. Previous research on CVD triggers has referred to the time period immediately following an infection as a treatable moment that may hold an opportunity for CVD prevention.[119] Previous work has identified infection as a particularly strong CVD trigger among those with otherwise low CVD risk.[118, 119, 199] CVD prophylaxis following an infection may be particularly relevant for these patients. Our results may indicate that patients with an inpatient infection may be of particular interest for CVD prophylaxis.

Although antibiotics have not been shown to prevent vascular events,[200-202] evidence-based infection control efforts such as influenza vaccination may be considered because of the ability to not only reduce infection but may also reduce CVD and VTE.[203, 204]

References

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the global burden of disease study 2010. *Lancet*. 2012;380:2095-2128
2. Mendis S, Davis S, Norrving B. Organizational update: The world health organization global status report on noncommunicable diseases 2014; one more landmark step in the combat against stroke and vascular disease. *Stroke; a journal of cerebral circulation*. 2015;46:e121-122
3. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics-2016 update: A report from the american heart association. *Circulation*. 2016;133:e38-e360
4. McKenna MT, Michaud CM, Murray CJ, Marks JS. Assessing the burden of disease in the united states using disability-adjusted life years. *American journal of preventive medicine*. 2005;28:415-423
5. Arnold AM, Psaty BM, Kuller LH, Burke GL, Manolio TA, Fried LP, et al. Incidence of cardiovascular disease in older americans: The cardiovascular health study. *Journal of the American Geriatrics Society*. 2005;53:211-218
6. Wattanakit K, Folsom AR, Chambless LE, Nieto FJ. Risk factors for cardiovascular event recurrence in the atherosclerosis risk in communities (aric) study. *American heart journal*. 2005;149:606-612
7. Fryar CD, Chen TC, Li X. Prevalence of uncontrolled risk factors for cardiovascular disease: United states, 1999-2010. *NCHS Data Brief*. 2012:1-8
8. Dahabreh IJ, Paulus JK. Association of episodic physical and sexual activity with triggering of acute cardiac events: Systematic review and meta-analysis. *Jama*. 2011;305:1225-1233
9. Guiraud V, Amor MB, Mas JL, Touze E. Triggers of ischemic stroke: A systematic review. *Stroke; a journal of cerebral circulation*. 2010;41:2669-2677
10. Hartge P. A dictionary of epidemiology, sixth edition. *American journal of epidemiology*. 2015
11. . *Health, united states, 2015: With special feature on racial and ethnic health disparities*. Hyattsville, MD; 2016.
12. Miller EC, Elkind MS. Infection and stroke: An update on recent progress. *Current neurology and neuroscience reports*. 2016;16:2
13. Ji YN, An L, Zhan P, Chen XH. Cytomegalovirus infection and coronary heart disease risk: A meta-analysis. *Mol Biol Rep*. 2012;39:6537-6546
14. Wu YP, Sun DD, Wang Y, Liu W, Yang J. Herpes simplex virus type 1 and type 2 infection increases atherosclerosis risk: Evidence based on a meta-analysis. *BioMed research international*. 2016;2016:2630865
15. Petta S, Maida M, Macaluso FS, Barbara M, Licata A, Craxi A, et al. Hepatitis c virus infection is associated with increased cardiovascular mortality: A meta-analysis of observational studies. *Gastroenterology*. 2016;150:145-155 e144; quiz e115-146

16. Olubamwo OO, Aregbesola AO, Miettola J, Kauhanen J, Tuomainen TP. Hepatitis c and risk of coronary atherosclerosis - a systematic review. *Public health*. 2016
17. Islam FM, Wu J, Jansson J, Wilson DP. Relative risk of cardiovascular disease among people living with hiv: A systematic review and meta-analysis. *HIV medicine*. 2012;13:453-468
18. Corrales-Medina VF, Alvarez KN, Weissfeld LA, Angus DC, Chirinos JA, Chang CC, et al. Association between hospitalization for pneumonia and subsequent risk of cardiovascular disease. *Jama*. 2015;313:264-274
19. Danesh J, Whincup P, Lewington S, Walker M, Lennon L, Thomson A, et al. Chlamydia pneumoniae iga titres and coronary heart disease; prospective study and meta-analysis. *European heart journal*. 2002;23:371-375
20. Smieja M, Mahony J, Petrich A, Boman J, Chernesky M. Association of circulating chlamydia pneumoniae DNA with cardiovascular disease: A systematic review. *BMC infectious diseases*. 2002;2:21
21. Su X, Chen HL. Chlamydia pneumoniae infection and cerebral infarction risk: A meta-analysis. *International journal of stroke : official journal of the International Stroke Society*. 2014;9:356-364
22. Hsiao LC, Muo CH, Chen YC, Chou CY, Tseng CH, Chang KC. Increased risk of coronary heart disease in patients with chronic osteomyelitis: A population-based study in a cohort of 23 million. *Heart*. 2014;100:1450-1454
23. Sun J, Rangan P, Bhat SS, Liu L. A meta-analysis of the association between helicobacter pylori infection and risk of coronary heart disease from published prospective studies. *Helicobacter*. 2016;21:11-23
24. Liu J, Wang F, Shi S. Helicobacter pylori infection increase the risk of myocardial infarction: A meta-analysis of 26 studies involving more than 20,000 participants. *Helicobacter*. 2015;20:176-183
25. Zhang S, Guo Y, Ma Y, Teng Y. Cytotoxin-associated gene-a-seropositive virulent strains of helicobacter pylori and atherosclerotic diseases: A systematic review. *Chinese medical journal*. 2008;121:946-951
26. Yu M, Zhang Y, Yang Z, Ding J, Xie C, Lu N. Association between helicobacter pylori infection and stroke: A meta-analysis of prospective observational studies. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association*. 2014;23:2233-2239
27. Wang ZW, Li Y, Huang LY, Guan QK, Xu DW, Zhou WK, et al. Helicobacter pylori infection contributes to high risk of ischemic stroke: Evidence from a meta-analysis. *Journal of neurology*. 2012;259:2527-2537
28. Caplan DJ. Epidemiologic issues in studies of association between apical periodontitis and systemic health. *Endodontic Topics*. 2004;8:15-35
29. Offenbacher S. Periodontal diseases: Pathogenesis. *Annals of periodontology / the American Academy of Periodontology*. 1996;1:821-878
30. Lockhart PB, Bolger AF, Papapanou PN, Osinbowale O, Trevisan M, Levison ME, et al. Periodontal disease and atherosclerotic vascular disease: Does the

evidence support an independent association?: A scientific statement from the american heart association. *Circulation*. 2012;125:2520-2544
31. Health NIo. Periodontal (gum) disease causes, symptoms, and treatments. 2013
32. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, et al. Update on prevalence of periodontitis in adults in the united states: Nhanes 2009 to 2012. *Journal of periodontology*. 2015;86:611-622
33. Borrell LN, Papapanou PN. Analytical epidemiology of periodontitis. *Journal of clinical periodontology*. 2005;32 Suppl 6:132-158
34. Ong G. Periodontal disease and tooth loss. *International dental journal*. 1998;48:233-238
35. Howell TH, Ridker PM, Ajani UA, Hennekens CH, Christen WG. Periodontal disease and risk of subsequent cardiovascular disease in u.S. Male physicians. *Journal of the American College of Cardiology*. 2001;37:445-450
36. Ryden L, Buhlin K, Ekstrand E, de Faire U, Gustafsson A, Holmer J, et al. Periodontitis increases the risk of a first myocardial infarction: A report from the parokrank study. *Circulation*. 2016;133:576-583
37. Beck JD, Eke P, Heiss G, Madianos P, Couper D, Lin D, et al. Periodontal disease and coronary heart disease: A reappraisal of the exposure. *Circulation*. 2005;112:19-24
38. Desvarieux M, Demmer RT, Rundek T, Boden-Albala B, Jacobs DR, Jr., Sacco RL, et al. Periodontal microbiota and carotid intima-media thickness: The oral infections and vascular disease epidemiology study (invest). *Circulation*. 2005;111:576-582
39. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. *Journal of periodontology*. 2012;83:1449-1454
40. Morelli T, Moss KL, Beck J, Preisser JS, Wu D, Divaris K, et al. Derivation and validation of the periodontal and tooth profile classification system for patient stratification. *Journal of periodontology*. 2016:1-17
41. Loesche WJ, Schork A, Terpenning MS, Chen YM, Dominguez BL, Grossman N. Assessing the relationship between dental disease and coronary heart disease in elderly u.S. Veterans. *Journal of the American Dental Association*. 1998;129:301-311
42. Arbes SJ, Jr., Slade GD, Beck JD. Association between extent of periodontal attachment loss and self-reported history of heart attack: An analysis of nhanes iii data. *Journal of dental research*. 1999;78:1777-1782
43. Katz J, Chaushu G, Sharabi Y. On the association between hypercholesterolemia, cardiovascular disease and severe periodontal disease. *Journal of clinical periodontology*. 2001;28:865-868
44. Buhlin K, Gustafsson A, Hakansson J, Klinge B. Oral health and cardiovascular disease in sweden. *Journal of clinical periodontology*. 2002;29:254-259

45. Malthaner SC, Moore S, Mills M, Saad R, Sabatini R, Takacs V, et al. Investigation of the association between angiographically defined coronary artery disease and periodontal disease. *Journal of periodontology*. 2002;73:1169-1176
46. Buhlin K, Gustafsson A, Hakansson J, Klinge B. Self-reported oral health, dental care habits and cardiovascular disease in an adult swedish population. *Oral health & preventive dentistry*. 2003;1:291-299
47. Frisk F, Hakeberg M, Ahlqwist M, Bengtsson C. Endodontic variables and coronary heart disease. *Acta odontologica Scandinavica*. 2003;61:257-262
48. Elter JR, Champagne CM, Offenbacher S, Beck JD. Relationship of periodontal disease and tooth loss to prevalence of coronary heart disease. *Journal of periodontology*. 2004;75:782-790
49. Holmlund A, Holm G, Lind L. Severity of periodontal disease and number of remaining teeth are related to the prevalence of myocardial infarction and hypertension in a study based on 4,254 subjects. *Journal of periodontology*. 2006;77:1173-1178
50. Gotsman I, Lotan C, Soskolne WA, Rassovsky S, Pugatsch T, Lapidus L, et al. Periodontal destruction is associated with coronary artery disease and periodontal infection with acute coronary syndrome. *Journal of periodontology*. 2007;78:849-858
51. Beukers NG, van der Heijden GJ, van Wijk AJ, Loos BG. Periodontitis is an independent risk indicator for atherosclerotic cardiovascular diseases among 60 174 participants in a large dental school in the netherlands. *Journal of epidemiology and community health*. 2016
52. Geerts SO, Legrand V, Charpentier J, Albert A, Rompen EH. Further evidence of the association between periodontal conditions and coronary artery disease. *Journal of periodontology*. 2004;75:1274-1280
53. Montebugnoli L, Servidio D, Miaton RA, Prati C, Tricoci P, Melloni C. Poor oral health is associated with coronary heart disease and elevated systemic inflammatory and haemostatic factors. *Journal of clinical periodontology*. 2004;31:25-29
54. Renvert S, Ohlsson O, Persson S, Lang NP, Persson GR. Analysis of periodontal risk profiles in adults with or without a history of myocardial infarction. *Journal of clinical periodontology*. 2004;31:19-24
55. Buhlin K, Gustafsson A, Ahnve S, Janszky I, Tabrizi F, Klinge B. Oral health in women with coronary heart disease. *Journal of periodontology*. 2005;76:544-550
56. Cueto A, Mesa F, Bravo M, Ocana-Riola R. Periodontitis as risk factor for acute myocardial infarction. A case control study of spanish adults. *Journal of periodontal research*. 2005;40:36-42
57. Andriankaja OM, Genco RJ, Dorn J, Dmochowski J, Hovey K, Falkner KL, et al. The use of different measurements and definitions of periodontal disease in the study of the association between periodontal disease and risk of myocardial infarction. *Journal of periodontology*. 2006;77:1067-1073

58. Briggs JE, McKeown PP, Crawford VL, Woodside JV, Stout RW, Evans A, et al. Angiographically confirmed coronary heart disease and periodontal disease in middle-aged males. *Journal of periodontology*. 2006;77:95-102
59. Geismar K, Stoltze K, Sigurd B, Gyntelberg F, Holmstrup P. Periodontal disease and coronary heart disease. *Journal of periodontology*. 2006;77:1547-1554
60. Rech RL, Nurkin N, da Cruz I, Sostizzo F, Baiao C, Perrone JA, et al. Association between periodontal disease and acute coronary syndrome. *Arquivos brasileiros de cardiologia*. 2007;88:185-190
61. Latronico M, Segantini A, Cavallini F, Mascolo A, Garbarino F, Bondanza S, et al. Periodontal disease and coronary heart disease: An epidemiological and microbiological study. *New Microbiol*. 2007;30:221-228
62. Barilli AL, Passos AD, Marin-Neto JA, Franco LJ. Periodontal disease in patients with ischemic coronary atherosclerosis at a university hospital. *Arquivos brasileiros de cardiologia*. 2006;87:695-700
63. Nonnenmacher C, Stelzel M, Susin C, Sattler AM, Schaefer JR, Maisch B, et al. Periodontal microbiota in patients with coronary artery disease measured by real-time polymerase chain reaction: A case-control study. *Journal of periodontology*. 2007;78:1724-1730
64. DeStefano F, Anda RF, Kahn HS, Williamson DF, Russell CM. Dental disease and risk of coronary heart disease and mortality. *Bmj*. 1993;306:688-691
65. Morrison HI, Ellison LF, Taylor GW. Periodontal disease and risk of fatal coronary heart and cerebrovascular diseases. *Journal of cardiovascular risk*. 1999;6:7-11
66. Jansson L, Lavstedt S, Frithiof L. Relationship between oral health and mortality rate. *Journal of clinical periodontology*. 2002;29:1029-1034
67. Tuominen R, Reunanen A, Paunio M, Paunio I, Aromaa A. Oral health indicators poorly predict coronary heart disease deaths. *Journal of dental research*. 2003;82:713-718
68. Hung HC, Joshipura KJ, Colditz G, Manson JE, Rimm EB, Speizer FE, et al. The association between tooth loss and coronary heart disease in men and women. *Journal of public health dentistry*. 2004;64:209-215
69. Abnet CC, Qiao YL, Dawsey SM, Dong ZW, Taylor PR, Mark SD. Tooth loss is associated with increased risk of total death and death from upper gastrointestinal cancer, heart disease, and stroke in a chinese population-based cohort. *International journal of epidemiology*. 2005;34:467-474
70. Cabrera C, Hakeberg M, Ahlqwist M, Wedel H, Bjorkelund C, Bengtsson C, et al. Can the relation between tooth loss and chronic disease be explained by socio-economic status? A 24-year follow-up from the population study of women in gothenburg, sweden. *European journal of epidemiology*. 2005;20:229-236
71. Mattila KJ, Valtonen VV, Nieminen M, Huttunen JK. Dental infection and the risk of new coronary events: Prospective study of patients with documented coronary artery disease. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 1995;20:588-592

72. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. *Journal of periodontology*. 1996;67:1123-1137
73. Joshipura KJ, Rimm EB, Douglass CW, Trichopoulos D, Ascherio A, Willett WC. Poor oral health and coronary heart disease. *Journal of dental research*. 1996;75:1631-1636
74. Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA. Periodontal disease and coronary heart disease risk. *Jama*. 2000;284:1406-1410
75. Dietrich T, Jimenez M, Krall Kaye EA, Vokonas PS, Garcia RI. Age-dependent associations between chronic periodontitis/edentulism and risk of coronary heart disease. *Circulation*. 2008;117:1668-1674
76. Mucci LA, Hsieh CC, Williams PL, Arora M, Adami HO, de Faire U, et al. Do genetic factors explain the association between poor oral health and cardiovascular disease? A prospective study among swedish twins. *American journal of epidemiology*. 2009;170:615-621
77. Holmlund A, Holm G, Lind L. Number of teeth as a predictor of cardiovascular mortality in a cohort of 7,674 subjects followed for 12 years. *Journal of periodontology*. 2010;81:870-876
78. Noguchi S, Toyokawa S, Miyoshi Y, Suyama Y, Inoue K, Kobayashi Y. Five-year follow-up study of the association between periodontal disease and myocardial infarction among japanese male workers: My health up study. *Journal of public health*. 2015;37:605-611
79. Reichert S, Schulz S, Benten AC, Lutze A, Seifert T, Schlitt M, et al. Periodontal conditions and incidence of new cardiovascular events among patients with coronary vascular disease. *Journal of clinical periodontology*. 2016
80. Yu YH, Chasman DI, Buring JE, Rose L, Ridker PM. Cardiovascular risks associated with incident and prevalent periodontal disease. *Journal of clinical periodontology*. 2015;42:21-28
81. Hansen GM, Egeberg A, Holmstrup P, Hansen PR. Relation of periodontitis to risk of cardiovascular and all-cause mortality (from a danish nationwide cohort study). *The American journal of cardiology*. 2016;118:489-493
82. Blaizot A, Vergnes JN, Nuwwareh S, Amar J, Sixou M. Periodontal diseases and cardiovascular events: Meta-analysis of observational studies. *International dental journal*. 2009;59:197-209
83. Leng WD, Zeng XT, Kwong JS, Hua XP. Periodontal disease and risk of coronary heart disease: An updated meta-analysis of prospective cohort studies. *International journal of cardiology*. 2015;201:469-472
84. Humphrey LL, Fu R, Buckley DI, Freeman M, Helfand M. Periodontal disease and coronary heart disease incidence: A systematic review and meta-analysis. *Journal of general internal medicine*. 2008;23:2079-2086
85. Mustapha IZ, Debrey S, Oladubu M, Ugarte R. Markers of systemic bacterial exposure in periodontal disease and cardiovascular disease risk: A systematic review and meta-analysis. *Journal of periodontology*. 2007;78:2289-2302

86. Bahekar AA, Singh S, Saha S, Molnar J, Arora R. The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: A meta-analysis. *American heart journal*. 2007;154:830-837
87. Khader YS, Albashaireh ZS, Alomari MA. Periodontal diseases and the risk of coronary heart and cerebrovascular diseases: A meta-analysis. *Journal of periodontology*. 2004;75:1046-1053
88. Janket SJ, Baird AE, Chuang SK, Jones JA. Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2003;95:559-569
89. Sfyroeras GS, Roussas N, Saleptsis VG, Argyriou C, Giannoukas AD. Association between periodontal disease and stroke. *Journal of vascular surgery*. 2012;55:1178-1184
90. Lafon A, Pereira B, Dufour T, Rigouby V, Giroud M, Bejot Y, et al. Periodontal disease and stroke: A meta-analysis of cohort studies. *European journal of neurology : the official journal of the European Federation of Neurological Societies*. 2014;21:1155-1161, e1166-1157
91. Leira Y, Seoane J, Blanco M, Rodriguez-Yanez M, Takkouche B, Blanco J, et al. Association between periodontitis and ischemic stroke: A systematic review and meta-analysis. *European journal of epidemiology*. 2016
92. Elter JR, Offenbacher S, Toole JF, Beck JD. Relationship of periodontal disease and edentulism to stroke/tia. *Journal of dental research*. 2003;82:998-1001
93. Sanchez-Siles M, Rosa-Salazar V, Camacho-Alonso F, Salazar-Sanchez N, Cozar-Hidalgo J. Association between periodontal disease and venous thromboembolic disease. *Quintessence international*. 2013;44:567-573
94. Caplan DJ, Pankow JS, Cai J, Offenbacher S, Beck JD. The relationship between self-reported history of endodontic therapy and coronary heart disease in the atherosclerosis risk in communities study. *Journal of the American Dental Association*. 2009;140:1004-1012
95. Siqueira JF, Jr. Endodontic infections: Concepts, paradigms, and perspectives. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002;94:281-293
96. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. *Crit Rev Oral Biol Med*. 2004;15:348-381
97. Rechenberg DK, Held U, Burgstaller JM, Bosch G, Attin T. Pain levels and typical symptoms of acute endodontic infections: A prospective, observational study. *BMC Oral Health*. 2016;16:61
98. Ray JJ, Kirkpatrick TC. Healing of apical periodontitis through modern endodontic retreatment techniques. *Gen Dent*. 2013;61:19-23
99. Huumonen S, Suominen AL, Vehkalahti MM. Prevalence of apical periodontitis in root filled teeth: Findings from a nationwide survey in finland. *Int Endod J*. 2016
100. Kirkevang LL, Vaeth M, Horsted-Bindslev P, Bahrami G, Wenzel A. Risk factors for developing apical periodontitis in a general population. *Int Endod J*. 2007;40:290-299

101. Liljestrand JM, Mantyla P, Paju S, Buhlin K, Kopra KA, Persson GR, et al. Association of endodontic lesions with coronary artery disease. *Journal of dental research*. 2016
102. Gomes MS, Hugo FN, Hilgert JB, Sant'Ana Filho M, Padilha DM, Simonsick EM, et al. Apical periodontitis and incident cardiovascular events in the baltimore longitudinal study of ageing. *Int Endod J*. 2016;49:334-342
103. An GK, Morse DE, Kunin M, Goldberger RS, Psoter WJ. Association of radiographically diagnosed apical periodontitis and cardiovascular disease: A hospital records-based study. *J Endod*. 2016;42:916-920
104. Joshipura KJ, Pitiphat W, Hung HC, Willett WC, Colditz GA, Douglass CW. Pulpal inflammation and incidence of coronary heart disease. *J Endod*. 2006;32:99-103
105. Pitiphat W, Garcia RI, Douglass CW, Joshipura KJ. Validation of self-reported oral health measures. *Journal of public health dentistry*. 2002;62:122-128
106. Gomes MS, Hugo FN, Hilgert JB, Padilha DM, Simonsick EM, Ferrucci L, et al. Validity of self-reported history of endodontic treatment in the baltimore longitudinal study of aging. *J Endod*. 2012;38:589-593
107. Caplan DJ, Chasen JB, Krall EA, Cai J, Kang S, Garcia RI, et al. Lesions of endodontic origin and risk of coronary heart disease. *Journal of dental research*. 2006;85:996-1000
108. Petersen J, Glassl EM, Nasseri P, Crismani A, Luger AK, Schoenherr E, et al. The association of chronic apical periodontitis and endodontic therapy with atherosclerosis. *Clin Oral Investig*. 2014;18:1813-1823
109. Costa TH, de Figueiredo Neto JA, de Oliveira AE, Lopes e Maia Mde F, de Almeida AL. Association between chronic apical periodontitis and coronary artery disease. *J Endod*. 2014;40:164-167
110. Willershausen I, Weyer V, Peter M, Weichert C, Kasaj A, Munzel T, et al. Association between chronic periodontal and apical inflammation and acute myocardial infarction. *Odontology*. 2014;102:297-302
111. Frisk F. Epidemiological aspects on apical periodontitis. Studies based on the prospective population study of women in goteborg and the population study on oral health in jonkoping, sweden. *Swed Dent J Suppl*. 2007:11-78, 11 p preceding table of contents
112. Segura-Egea JJ, Jimenez-Moreno E, Calvo-Monroy C, Rios-Santos JV, Velasco-Ortega E, Sanchez-Dominguez B, et al. Hypertension and dental periapical condition. *J Endod*. 2010;36:1800-1804
113. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. *The New England journal of medicine*. 2004;351:2611-2618
114. Dalager-Pedersen M, Sogaard M, Schonheyder HC, Nielsen H, Thomsen RW. Risk for myocardial infarction and stroke after community-acquired bacteremia: A 20-year population-based cohort study. *Circulation*. 2014;129:1387-1396

115. Warren-Gash C, Hayward AC, Hemingway H, Denaxas S, Thomas SL, Timmis AD, et al. Influenza infection and risk of acute myocardial infarction in england and wales: A caliber self-controlled case series study. *The Journal of infectious diseases*. 2012;206:1652-1659
116. Guan X, Yang W, Sun X, Wang L, Ma B, Li H, et al. Association of influenza virus infection and inflammatory cytokines with acute myocardial infarction. *Inflamm Res*. 2012;61:591-598
117. Chew DP, Mattschoss S, Horsfall M, Astley C, Vaile JC, Joseph MX. Patterns of inflammatory activation associated with precipitants of acute coronary syndromes: A case-crossover study. *Internal medicine journal*. 2012;42:1096-1103
118. Cowan LT, Alonso A, Pankow JS, Folsom AR, Rosamond WD, Gottesman RF, et al. Hospitalized infection as a trigger for acute ischemic stroke: The atherosclerosis risk in communities study. *Stroke; a journal of cerebral circulation*. 2016;47:1612-1617
119. Elkind MS, Carty CL, O'Meara ES, Lumley T, Lefkowitz D, Kronmal RA, et al. Hospitalization for infection and risk of acute ischemic stroke: The cardiovascular health study. *Stroke; a journal of cerebral circulation*. 2011;42:1851-1856
120. Fullerton HJ, Hills NK, Elkind MS, Dowling MM, Wintermark M, Glaser CA, et al. Infection, vaccination, and childhood arterial ischemic stroke: Results of the vips study. *Neurology*. 2015;85:1459-1466
121. Cowan LT, Lutsey PL, Pankow JS, Cushman M, Folsom AR. Hospitalization with infection and incident venous thromboembolism: The aric study. *Thrombosis research*. 2017;151:74-78
122. Dalager-Pedersen M, Sogaard M, Schonheyder HC, Thomsen RW, Baron JA, Nielsen H. Venous thromboembolism after community-acquired bacteraemia: A 20-year danish cohort study. *PloS one*. 2014;9:e86094
123. Masrouha KZ, Musallam KM, Rosendaal FR, Hoballah JJ, Jamali FR. Preoperative pneumonia and postoperative venous thrombosis: A cohort study of 427,656 patients undergoing major general surgery. *World journal of surgery*. 2016
124. Rogers MA, Levine DA, Blumberg N, Flanders SA, Chopra V, Langa KM. Triggers of hospitalization for venous thromboembolism. *Circulation*. 2012;125:2092-2099
125. Chen YG, Lin TY, Huang WY, Lin CL, Dai MS, Kao CH. Association between pneumococcal pneumonia and venous thromboembolism in hospitalized patients: A nationwide population-based study. *Respirology*. 2015;20:799-804
126. Demmer RT, Desvarieux M. Periodontal infections and cardiovascular disease: The heart of the matter. *Journal of the American Dental Association*. 2006;137 Suppl:14S-20S; quiz 38S
127. Chiu B. Multiple infections in carotid atherosclerotic plaques. *American heart journal*. 1999;138:S534-536

128. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. *Journal of periodontology*. 2000;71:1554-1560
129. Fong IW. Infections and their role in atherosclerotic vascular disease. *Journal of the American Dental Association*. 2002;133 Suppl:7S-13S
130. Esenwa CC, Elkind MS. Inflammatory risk factors, biomarkers and associated therapy in ischaemic stroke. *Nature reviews. Neurology*. 2016;12:594-604
131. Filardo S, Di Pietro M, Farcomeni A, Schiavoni G, Sessa R. Chlamydia pneumoniae-mediated inflammation in atherosclerosis: A meta-analysis. *Mediators Inflamm*. 2015;2015:378658
132. Vos AG, Idris NS, Barth RE, Klipstein-Grobusch K, Grobbee DE. Pro-inflammatory markers in relation to cardiovascular disease in hiv infection. A systematic review. *PloS one*. 2016;11:e0147484
133. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *Journal of periodontology*. 2000;71:1528-1534
134. D'Aiuto F, Parkar M, Andreou G, Suvan J, Brett PM, Ready D, et al. Periodontitis and systemic inflammation: Control of the local infection is associated with a reduction in serum inflammatory markers. *Journal of dental research*. 2004;83:156-160
135. Hannigan E, O'Connell DP, Hannigan A, Buckley LA. Soluble cell adhesion molecules in gingival crevicular fluid in periodontal health and disease. *Journal of periodontology*. 2004;75:546-550
136. Freitas CO, Gomes-Filho IS, Naves RC, Nogueira Filho Gda R, Cruz SS, Santos CA, et al. Influence of periodontal therapy on c-reactive protein level: A systematic review and meta-analysis. *J Appl Oral Sci*. 2012;20:1-8
137. Gomes MS, Blattner TC, Sant'Ana Filho M, Grecca FS, Hugo FN, Fouad AF, et al. Can apical periodontitis modify systemic levels of inflammatory markers? A systematic review and meta-analysis. *J Endod*. 2013;39:1205-1217
138. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, c-reactive protein, albumin, or leukocyte count with coronary heart disease: Meta-analyses of prospective studies. *Jama*. 1998;279:1477-1482
139. Hernandez-Diaz Y, Tovilla-Zarate CA, Juarez-Rojop I, Banos-Gonzalez MA, Torres-Hernandez ME, Lopez-Narvaez ML, et al. The role of gene variants of the inflammatory markers crp and tnf-alpha in cardiovascular heart disease: Systematic review and meta-analysis. *Int J Clin Exp Med*. 2015;8:11958-11984
140. Kaptoge S, Seshasai SR, Gao P, Freitag DF, Butterworth AS, Borglykke A, et al. Inflammatory cytokines and risk of coronary heart disease: New prospective study and updated meta-analysis. *European heart journal*. 2014;35:578-589
141. Emerging Risk Factors C, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: An individual participant meta-analysis. *Lancet*. 2010;375:132-140

142. Folsom AR, Lutsey PL, Astor BC, Cushman M. C-reactive protein and venous thromboembolism. A prospective investigation in the aric cohort. *Thrombosis and haemostasis*. 2009;102:615-619
143. Awan Z, Genest J. Inflammation modulation and cardiovascular disease prevention. *European journal of preventive cardiology*. 2015;22:719-733
144. Emerging Risk Factors C, Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *The New England journal of medicine*. 2012;367:1310-1320
145. McKibben RA, Haberlen SA, Post WS, Brown TT, Budoff M, Witt MD, et al. A cross-sectional study of the association between chronic hepatitis c virus infection and subclinical coronary atherosclerosis among participants in the multicenter aids cohort study. *The Journal of infectious diseases*. 2016;213:257-265
146. Burgner DP, Sabin MA, Magnussen CG, Cheung M, Sun C, Kahonen M, et al. Early childhood hospitalisation with infection and subclinical atherosclerosis in adulthood: The cardiovascular risk in young finns study. *Atherosclerosis*. 2015;239:496-502
147. Cotti E, Dessi C, Piras A, Flore G, Deidda M, Madeddu C, et al. Association of endodontic infection with detection of an initial lesion to the cardiovascular system. *J Endod*. 2011;37:1624-1629
148. Huang H, Kang R, Zhao Z. Is hepatitis c associated with atherosclerotic burden? A systematic review and meta-analysis. *PloS one*. 2014;9:e106376
149. Zeng XT, Leng WD, Lam YY, Yan BP, Wei XM, Weng H, et al. Periodontal disease and carotid atherosclerosis: A meta-analysis of 17,330 participants. *International journal of cardiology*. 2016;203:1044-1051
150. Orlandi M, Suvan J, Petrie A, Donos N, Masi S, Hingorani A, et al. Association between periodontal disease and its treatment, flow-mediated dilatation and carotid intima-media thickness: A systematic review and meta-analysis. *Atherosclerosis*. 2014;236:39-46
151. Teeuw WJ, Slot DE, Susanto H, Gerdes VE, Abbas F, D'Aiuto F, et al. Treatment of periodontitis improves the atherosclerotic profile: A systematic review and meta-analysis. *Journal of clinical periodontology*. 2014;41:70-79
152. Liang H, Duan Z, Li D, Li D, Wang Z, Ren L, et al. Higher levels of circulating monocyte-platelet aggregates are correlated with viremia and increased scd163 levels in hiv-1 infection. *Cell Mol Immunol*. 2015;12:435-443
153. Perumal R, Rajendran M, Krishnamurthy M, Ganji KK, Pendor SD. Modulation of p-selection and platelet aggregation in chronic periodontitis: A clinical study. *J Indian Soc Periodontol*. 2014;18:293-300
154. Banthia R, Jain P, Banthia P, Belludi S, Parwani S, Jain A. Effect of phase i periodontal therapy on pro-coagulant state in chronic periodontitis patients--a clinical and haematological study. *J Ir Dent Assoc*. 2013;59:183-188
155. Roth GA, Moser B, Huang SJ, Brandt JS, Huang Y, Papapanou PN, et al. Infection with a periodontal pathogen induces procoagulant effects in human

aortic endothelial cells. *Journal of thrombosis and haemostasis : JTH*. 2006;4:2256-2261
156. Herzberg MC, Meyer MW. Effects of oral flora on platelets: Possible consequences in cardiovascular disease. *Journal of periodontology*. 1996;67:1138-1142
157. Meyer MW, Gong K, Herzberg MC. Streptococcus sanguis-induced platelet clotting in rabbits and hemodynamic and cardiopulmonary consequences. *Infection and immunity*. 1998;66:5906-5914
158. The atherosclerosis risk in communities (aric) study: Design and objectives. The aric investigators. *American journal of epidemiology*. 1989;129:687-702
159. White AD, Folsom AR, Chambless LE, Sharret AR, Yang K, Conwill D, et al. Community surveillance of coronary heart disease in the atherosclerosis risk in communities (aric) study: Methods and initial two years' experience. *Journal of clinical epidemiology*. 1996;49:223-233
160. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Polak JF, Folsom AR. Cardiovascular risk factors and venous thromboembolism incidence: The longitudinal investigation of thromboembolism etiology. *Archives of internal medicine*. 2002;162:1182-1189
161. Statistics NCfH. Us department of health and human services, nhanes iii reference manuals and reports 1996
162. Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S. Relationship of periodontal disease to carotid artery intima-media wall thickness: The atherosclerosis risk in communities (aric) study. *Arteriosclerosis, thrombosis, and vascular biology*. 2001;21:1816-1822
163. Altman D, Frist WH. Medicare and medicaid at 50 years: Perspectives of beneficiaries, health care professionals and institutions, and policy makers. *Jama*. 2015;314:384-395
164. Kucharska-Newton AM, Heiss G, Ni H, Stearns SC, Puccinelli-Ortega N, Wruck LM, et al. Identification of heart failure events in medicare claims: The atherosclerosis risk in communities (aric) study. *Journal of cardiac failure*. 2016;22:48-55
165. Libby P. Inflammation and cardiovascular disease mechanisms. *The American journal of clinical nutrition*. 2006;83:456S-460S
166. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *The New England journal of medicine*. 2000;342:836-843
167. Centers for Disease C, Prevention. Venous thromboembolism in adult hospitalizations - united states, 2007-2009. *MMWR. Morbidity and mortality weekly report*. 2012;61:401-404
168. Cushman M, Tsai AW, White RH, Heckbert SR, Rosamond WD, Enright P, et al. Deep vein thrombosis and pulmonary embolism in two cohorts: The longitudinal investigation of thromboembolism etiology. *The American journal of medicine*. 2004;117:19-25

169. Anderson FA, Jr., Spencer FA. Risk factors for venous thromboembolism. *Circulation*. 2003;107:I9-16
170. Mahmoodi BK, Cushman M, Anne Naess I, Allison MA, Jan Bos W, Braekkan SK, et al. Association of traditional cardiovascular risk factors with venous thromboembolism: An individual participant data meta-analysis of prospective studies. *Circulation*. 2017;135:7-16
171. Fiorini T, Musskopf ML, Oppermann RV, Susin C. Is there a positive effect of smoking cessation on periodontal health? A systematic review. *Journal of periodontology*. 2014;85:83-91
172. Gaborit FS, Overvad K, Norgaard M, Kristensen SR, Tjonneland A, Severinsen MT. Alcohol intake and risk of venous thromboembolism. A danish follow-up study. *Thrombosis and haemostasis*. 2013;110:39-45
173. Shepherd S. Alcohol consumption a risk factor for periodontal disease. *Evid Based Dent*. 2011;12:76
174. Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW. Cardiovascular risk factors and venous thromboembolism: A meta-analysis. *Circulation*. 2008;117:93-102
175. Chaffee BW, Weston SJ. Association between chronic periodontal disease and obesity: A systematic review and meta-analysis. *Journal of periodontology*. 2010;81:1708-1724
176. Ashrani AA, Barsoum MK, Crusan DJ, Petterson TM, Bailey KR, Heit JA. Is lipid lowering therapy an independent risk factor for venous thromboembolism? A population-based case-control study. *Thrombosis research*. 2015;135:1110-1116
177. Subramanian S, Emami H, Vucic E, Singh P, Vijayakumar J, Fifer KM, et al. High-dose atorvastatin reduces periodontal inflammation: A novel pleiotropic effect of statins. *Journal of the American College of Cardiology*. 2013;62:2382-2391
178. Dupont WD, Plummer WD, Jr. Power and sample size calculations. A review and computer program. *Controlled clinical trials*. 1990;11:116-128
179. Dupont WD, Plummer WD, Jr. Power and sample size calculations for studies involving linear regression. *Controlled clinical trials*. 1998;19:589-601
180. Li C, Lv Z, Shi Z, Zhu Y, Wu Y, Li L, et al. Periodontal therapy for the management of cardiovascular disease in patients with chronic periodontitis. *The Cochrane database of systematic reviews*. 2014:CD009197
181. Narayanan LL, Vaishnavi C. Endodontic microbiology. *J Conserv Dent*. 2010;13:233-239
182. Nibali L, Fedele S, D'Aiuto F, Donos N. Interleukin-6 in oral diseases: A review. *Oral diseases*. 2012;18:236-243
183. Barkhordar RA, Hayashi C, Hussain MZ. Detection of interleukin-6 in human dental pulp and periapical lesions. *Endod Dent Traumatol*. 1999;15:26-27

184. Garrido M, Dezerega A, Bordagaray MJ, Reyes M, Vernal R, Melgar-Rodriguez S, et al. C-reactive protein expression is up-regulated in apical lesions of endodontic origin in association with interleukin-6. *J Endod*. 2015;41:464-469
185. Cotti E, Mercuro G. Apical periodontitis and cardiovascular diseases: Previous findings and ongoing research. *Int Endod J*. 2015;48:926-932
186. Berlin-Broner Y, Febbraio M, Levin L. Association between apical periodontitis and cardiovascular diseases: A systematic review of the literature. *Int Endod J*. 2016
187. Hardy DS, Hoelscher DM, Aragaki C, Stevens J, Steffen LM, Pankow JS, et al. Association of glycemic index and glycemic load with risk of incident coronary heart disease among whites and african americans with and without type 2 diabetes: The atherosclerosis risk in communities study. *Annals of epidemiology*. 2010;20:610-616
188. Rosamond WD, Folsom AR, Chambless LE, Wang CH, McGovern PG, Howard G, et al. Stroke incidence and survival among middle-aged adults: 9-year follow-up of the atherosclerosis risk in communities (aric) cohort. *Stroke; a journal of cerebral circulation*. 1999;30:736-743
189. Loehr LR, Rosamond WD, Chang PP, Folsom AR, Chambless LE. Heart failure incidence and survival (from the atherosclerosis risk in communities study). *The American journal of cardiology*. 2008;101:1016-1022
190. Segura-Egea JJ, Castellanos-Cosano L, Velasco-Ortega E, Rios-Santos JV, Llamas-Carreras JM, Machuca G, et al. Relationship between smoking and endodontic variables in hypertensive patients. *J Endod*. 2011;37:764-767
191. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart disease and stroke statistics-2017 update: A report from the american heart association. *Circulation*. 2017;135:e146-e603
192. Slutzky-Goldberg I, Baev V, Volkov A, Zini A, Tsesis I. Incidence of cholesterol in periapical biopsies among adolescent and elderly patients. *J Endod*. 2013;39:1477-1480
193. Wang CH, Chueh LH, Chen SC, Feng YC, Hsiao CK, Chiang CP. Impact of diabetes mellitus, hypertension, and coronary artery disease on tooth extraction after nonsurgical endodontic treatment. *J Endod*. 2011;37:1-5
194. Martin-Cabezas R, Seelam N, Petit C, Agossa K, Gaertner S, Tenenbaum H, et al. Association between periodontitis and arterial hypertension: A systematic review and meta-analysis. *American heart journal*. 2016;180:98-112
195. Caula AL, Lira-Junior R, Tinoco EM, Fischer RG. The effect of periodontal therapy on cardiovascular risk markers: A 6-month randomized clinical trial. *Journal of clinical periodontology*. 2014;41:875-882
196. Jansson L, Lavstedt S, Frithiof L, Theobald H. Relationship between oral health and mortality in cardiovascular diseases. *Journal of clinical periodontology*. 2001;28:762-768

197. Nawrot TS, Perez L, Kunzli N, Munters E, Nemery B. Public health importance of triggers of myocardial infarction: A comparative risk assessment. *Lancet*. 2011;377:732-740
198. Epaulard O, Foote A, Bosson JL. Chronic infection and venous thromboembolic disease. *Seminars in thrombosis and hemostasis*. 2015
199. Levine DA, Langa KM, Rogers MA. Acute infection contributes to racial disparities in stroke mortality. *Neurology*. 2014;82:914-921
200. Muhlestein JB, Anderson JL, Carlquist JF, Salunkhe K, Horne BD, Pearson RR, et al. Randomized secondary prevention trial of azithromycin in patients with coronary artery disease: Primary clinical results of the academic study. *Circulation*. 2000;102:1755-1760
201. Vainas T, Stassen FR, Schurink GW, Tordoir JH, Welten RJ, van den Akker LH, et al. Secondary prevention of atherosclerosis through chlamydia pneumoniae eradication (space trial): A randomised clinical trial in patients with peripheral arterial disease. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*. 2005;29:403-411
202. Grayston JT, Kronmal RA, Jackson LA, Parisi AF, Muhlestein JB, Cohen JD, et al. Azithromycin for the secondary prevention of coronary events. *The New England journal of medicine*. 2005;352:1637-1645
203. Udell JA, Zawi R, Bhatt DL, Keshtkar-Jahromi M, Gaughran F, Phrommintikul A, et al. Association between influenza vaccination and cardiovascular outcomes in high-risk patients: A meta-analysis. *Jama*. 2013;310:1711-1720
204. Zhu T, Carcaillon L, Martinez I, Cambou JP, Kyndt X, Guillot K, et al. Association of influenza vaccination with reduced risk of venous thromboembolism. *Thrombosis and haemostasis*. 2009;102:1259-1264